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Angiotensin-converting enzyme gene insertion/deletion (I/D) polymorphism in Azerbaijan population

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Angiotensin-converting enzyme (ACE) is a key enzyme of the renin-angiotensin-aldosterone system (RAAS), which is directly involved in the regulation of blood pressure. It is assumed that the insertion/deletion (I/D) polymorphism of the gene of this enzyme (ACE gene) appears due to the presence/absence of ~ 287 bp Alu repeats in the 16th intron and is associated with the risk of the development of some diseases, including cardiovascular diseases, various kinds of mental disorders, Alzheimer's disease, gestational diabetes, etc. Given the lack of data on ACE gene I/D polymorphism for the Azerbaijan population, we studied polymorphism of this gene by PCR, using sequence specific pairs of primers (Hace3s and Hace3as (I), ACE-F and ACE-R (II)). DNA samples isolated from 346 individuals were divided into 4 groups: (1) patients with various mental disorders (90 patients); (2) a group of young students involved in various sports (84 male persons); (3) patients with diabetes (28 patients with I type DM (3A subgroup) and 72 patients with II type DM (3B subgroup); (4) a group of conditionally healthy people of different ages and specialties (72 persons, control). Based on the results of PCR of both primer pairs, the following genotypes were obtained: 16 individuals with genotype II (4.6%, homozygous co-dominants for the I-allele), 101 individuals with genotype DD (29.2%, homozygous co-dominants for the D-allele) and 228 individuals with genotype ID (66.2%, heterozygotes for both alleles). The frequency of occurrence was: f_I=0.373, f_D=0.627, N_D:N_I=1.681. The ratio of separate genotypes within the studied population: ID:DD=2.173; ID:II=14.125; DD:II=6.500. Comparison of the values of the dominant model for the allele D - (DD+ID)/II=20.625 and the recessive model DD/(ID+II)=0.430 relative to the dominant model for the allele I - (II+ID)/DD=1.152 and the recessive model II/(ID+DD)=0.048 indicates that in both models the probability of the D allele to associate with any particular trait is higher than that of the I allele (17.904 and 8.958 times, respectively). These results confirm the literature data on the association of the D allele with many pathologies or diseases. The analysis of the obtained data also revealed a significant correlation ($p \leq 0.01$) of the studied features from the D allele both within groups and between groups.

Keywords: *Renin-angiotensin-aldosterone system (RAAS), angiotensin-converting enzyme (ACE), insertion, deletion, hypertension, psychiatric disorders, diabetes mellitus (DM), polymorphism, co-dominant, homozygote, heterozygote, COVID-19*

INTRODUCTION

The renin-angiotensin-aldosterone system (RAAS) which include renin inhibitors, angiotensin-converting enzyme (ACE) inhibitors, angiotensin II type 1 receptor antagonists, and mineralocorticoid receptor antagonists, is known as a regulator of hypertension and fluid as well as electrolyte homeostasis. ACE is a key enzyme in the RAAS that is converting angiotensin I to the vasoactive peptide angiotensin II. Renin (EC 3.4.99.19), the enzyme that catalyzes the proteolytic conversion of angiotensinogen to the decapeptide angiotensin I (Khakoo et al., 2008) Angiotensinogen, a large

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globular protein that serves as the substrate for renin (Malikova et al., 2016). Angiotensin II and the angiotensin II receptor, control the transduction of the cellular effects of angiotensin II (Forrester et al., 2018). Binding of the angiotensin II to its receptor mediates vasoconstriction, aldosterone and catecholamine release, as well as fluid consumption, secretion of prolactin, adrenocorticotrophic hormone, and glycogenolysis (Lynch et al., 2018).

The four major components of the RAAS (renin, angiotensinogen [angiotensins I and II], angiotensin-converting enzyme, and the AT receptors) are important components of vascular diseases (De Mello et al., 2017; Lynch et al., 2018).

ACE was the first discovered in the 1950s by Skeggs and colleagues. This enzyme was able to convert angiotensin I to angiotensin II (called hypertensin I and hypertensin II at that time) in horse plasma. ACE was originaly called "converting enzyme". Later Phillips and colleagues identified an enzyme in human blood, designated kininase II, which was able to degrade bradykinin (Phillips et al., 2018). Converting enzyme and kininase II was later found to be the same enzyme and today the enzyme is referred to as angiotensin-converting enzyme. ACE is a dipeptidyl carboxypeptidase that exists in 2 isoforms. Somatic ACE is induced in different tissues and cell types including the cardiovascular system, lungs, kidneys, etc, whereas testicular ACE only can be found in sperm cells. Both isoforms have a hydrophobic trans-membrane domain and a short cytoplasmic fragment (De Mello et al., 2017). The ACE gene is located in chromosome 17 (17q23 region) and contains a polymorphism based on the presence (insertion) or absence (deletion) of a 287 base-pair (bp) fragment on 16th intron of ACE gene. Based on the polymorphism there are three genotypes; ID, II, DD which are classified as I and D alleles which are termed as insertion or deletion, respectively (Turgut et al., 2004) .

ACE I/D polymorphism associated to the level of ACE in plasma and with higher risk for cardiovascular diseases. Three ACE genotypes – II, ID, and DD, have different correlation with the percentage of hypertension, myocardial hypertrophy, diabetes mellitus, psychological disorders and other diseases. The D allele has been connected with an increased risk of developing various pathological processes, such as coronary heart disease and ventricular hypertrophy. While various cardiac disorders appear to have a worse prognosis in individuals homozygous for the D allele, the I allele has been associated with increased endurance performance in athletes (Goessler et al., 2016). Polymorphisms of the ACE gene was found to be involved in bipolar disorders as well as in schizophrenia (Song et al., 2015). Bipolar disorders are severe early-onset diseases that comprise psychiatric conditions characterized by recurrent mood changes from depression to mania. Their prevalence is high, possibly as much as 5%, in the US population (Dal Mas et al., 2019). The presence of the I/D genotype of the ACE gene contribute to an increase of ACE plasma activity, which could be a predictor in schizophrenia diagnosis (Dal Mas et al., 2019; Gadelha et al., 2015).

The results of the polymorphism of the ACE gene in American 45 male football athletes vs 72 non-athletes showed a greater frequency of the D allele in athletes with comparison in non-athletes (Santoro et al., 2019). Moreover, a significant difference ($p \leq 0.05$) in the genotypic distribution of the athletes was composed of a higher number of the DD genotype as compared to the control group. However, the association of the I/D polymorphism of the ACE in sports abilities have been debated.

ACE gene I/D polymorphism is also associated with the development of diabetes mellitus (basically type II DM), gestasional diabetes and various comorbidities in different world populations (Purnamasari et al., 2012; Pan et al., 2016; Mir-Feizi et al., 2018; Pirozzi et al., 2018; Shen et al., 2019).

The new coronavirus COVID-19 (or Severe Acute Respiratory Syndrome SARS-CoV-2) which activated by transmembrane proteins (for example by transmembrane protease serine 2 (TMPRSS2)) mainly penetrates into the cells (endocytosis) through a receptors by binding to membranal ACE-2 widely expressed in cardiac cells including endothelial cells, smooth muscle cells in the myocardial vasculature and in cardiac myocytes (Fig. 1, for detailed see: Abbasi et al., 2020).

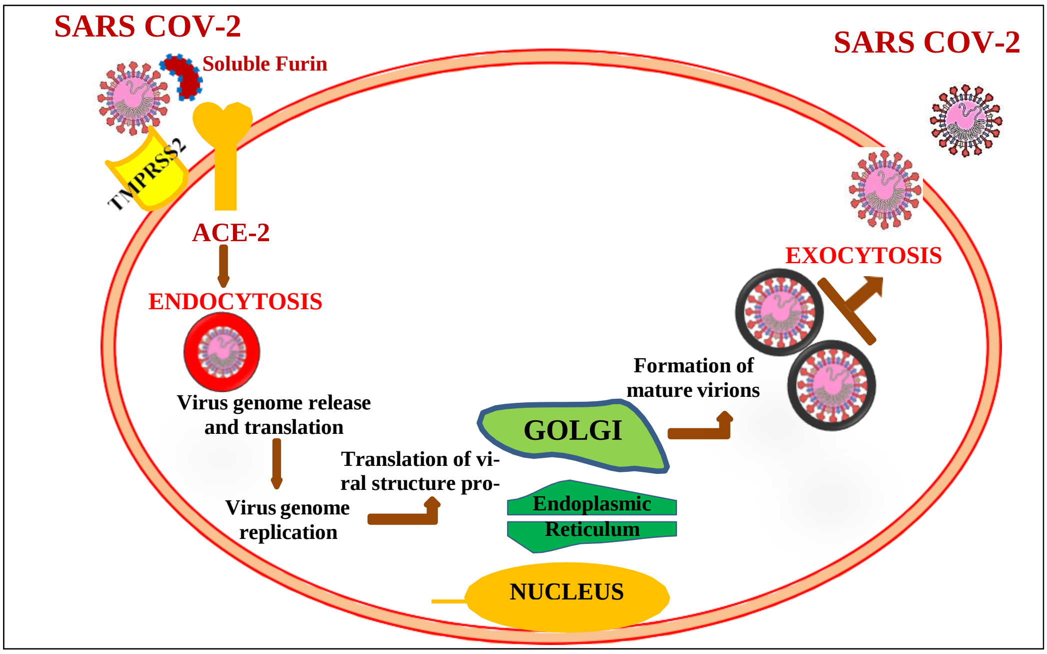


Fig. 1. The simplified scheme of the processes at the invasion of SARS-CoV-2.

Almost all cells, particularly cells of the epithelium and diaphragm of the lungs, which are more sensitive and accessible to the penetration of coronavirus, contain many of such receptors belonging to various forms and classes of ACE. In the way to produce an approaches for the treatment and prevention of infected by coronavirus patients, it is required to know all possible mechanisms of endocytosis that are directly or indirectly associated with both ACE receptor and ACE gene polymorphism (Das et al., 2020).

Coronavirus infection has a particularly high risk of fatalities with co-presence a number of autoimmune and chronic diseases (diabetes mellitus (I and II types), hypertension, chronic obstructive pulmonary disease, cardiovascular disease, cancer, etc.) as well as in seasonal respiratory diseases (influenza, inflammation, etc.) (Abbasi et al., 2020; Bosso et al., 2020; Brojakowska et al., 2020; Cure E., Cumhur Cure M., 2020; Devaux et al., 2020; Guo et al., 2020; Hussain et al., 2020; Li et al., 2020; Lippi et al., 2020; Othman et al., 2020; Öztürk et al., 2020; South et al., 2020; Yamamoto et al., 2020; Zhang et al., 2020). The investigation of I/D polymorphism of ACE gene may help to understand molecular mechanisms of coronovirus infection. However, I/D polymorphism of ACE gene in the Azerbaijan population has not been studied yet. Therefore, in this work, we study the ACE gene I/D polymorphism in the Azerbaijan population with various primers to clarify the association of these polymorphism with different diseases.

MATERIALS AND METHODS

Population Studied. The fresh blood samples were collected on a voluntary basis from 346 citizens of the republic in different ages and with different professional activities. The studied population sample include following groups:

(1) 84 male atlethes engaged in various sports; (2) 90 menthal pacients with various diagnoses (24 female, 66 male); (3) 100 pacients with diabetes mellitus (28 pacients I type DM (11 male and 17 female); 72 patients II type DM (21 male and 51 female)); (4) 72 condiotionally healthy individuals (control group, 42 female, 30 male).

DNA isolation procedures. DNA from 200 µl blood samples was isolated using "Diatom™ DNA Prep 200" kit (Izogen, Russian Rederation) on manufacturer protocols. DNA samples were stored at -80ºC. The concentrations and purity of the DNA samples were determined spectrophotometrically in Epoch™ Microplate Spectrophotometr (BioTek,

Aglient, USA) using Gene5 software. DNA samples were dilited individually before PCR.

Detection of I/D Polymorphism of ACE gene. ACE polymorphism on 16th intron was determined by polymerase chain reaction (PCR) using two pairs of specific primers: Hace3s (5'-GCCCTGCAGGTGTCTGCAGCATGT-3') and Hace3as (5'-GGATGGCTCTCCCCGCCTTGTC TC-3') (Castellano et al., 1995), ACE-F (5'-CTG-GAGACCACTCCCATCCTTTCT-3') and ACE-R (5'-GATGTGGCCATCACATTCGTCA GAT-3') (Rigat et al., 1992).

The obtained DNA fragments were electrophoresed in a 1.5% agarose gel and visualized by ethidium bromide staining. The sizes of fragments were estimated by comparison with previously known molecular weight markers M100. The polymorphism detected by PCR was evident as a 490-bp and 597 bp fragments in the presence of the insertion (I allele) and as a 190-bp and 319 bp product in the absence of the insertion (D allele). Each sample found to have the D/D genotype was subjected to a second PCR amplification with insertion-specific primers (5a: 5′-TGGGAC-CACAGCGCCCGCCACTAC-3′ and 5c: 5′-TCGCCAGCCCTCCCATGCCCA TAA-3′) in order to avoid D/D mistyping (Shanmugam et al., 1993).

RESULTS AND DISCUSSION

Primers Hace3s and Hace3as (Figure 2) revealed an insertion-specific 597 bp fragment in 16 samples, deletion-specific 319 bp fragment in 104 samples and insertion-deletion fragments of both types in 226 samples. Similar results were observed with the primer pair ACE-F and ACE-R (Figure 3), which yielded the corresponding 190 (with deletion) and 490 (with insertion) b.p. fragments.

Interestingly, the primer pair ACE-5a and ACE-5c, which synthesizes a specific 335 bp fragment, gave a positive result in only one case. Thus, we show that the allelic forms of the ACE gene for the Azerbaijan population of 346 individuals, consists of 66.2% of the ID, 29.2% of the DD and 4.6% carriers of the II genotype. Genotypes revealed among studied groups shown in Table 1.

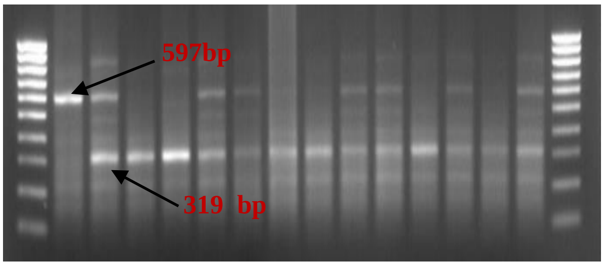


Fig 2. The amplification results with the Hace3s and Hace3as primers

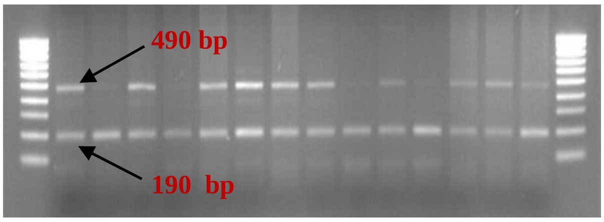


Fig 3. The amplification results with the ACE-F and ACE-R primers

Table 1. Genotypes revealed among studied groups

Groups, gender (M – male; F - female)			Genotypes II	ID	DD
1		M	6	45	15
		F	3	18	3
		Total:	**9**	**63**	**18**
2		M	-	63	21
		F	-	-	-
		Total:	**0**	**63**	**21**
3	**I type DM (3A)**	M	-	9	2
		F	1	9	7
	II type DM (3B)	M	-	12	9
		F	-	33	18
		Total:	**1**	**63**	**36**
4		M	-	15	12
		F	6	21	18
		Total:	**6**	**36**	**30**
Total on studied population		**M**	**6**	**144**	**59**
		F	**10**	**81**	**46**
			16	**225**	**105**

Note: – indicates the absence of given genotype.

ID heterozygous can be observed incorrectly by the PCR method (Shanmugam et al., 1993). Thus, the initial amplification of the shorter D allele during the studies leads to incorrect classification of approximately 4–5% of ID genotypes as DD. An additional PCR-based amplification reaction developed to confirm all DD genotypes obtained from the initial standart PCR to prevent any ID genotype be-

ing misidentified. This confirmatory results obtained in the classification of ACE polymorphism by the insertion-specific PCR method. The combined use of standard and confirmatory PCR methods used in numerous studies investigating the association of DD genotype with the diseases. Based on the numerous literature data (Gatt et al., 2015, Mengesha et al., 2019; Shen et al., 2019; Zhang et al., 2019) on the meta-analysis of the ACE gene polymorphism, the models in which the alleles D and I are dominant or recessive can be observed. The dominant model of the D allele (DD+ID)/II=20.625, the recessive model of the D allele DD/(ID+II)=0.430; The dominant model of the I allele (II+DD)/DD=1.152, the recessive model of the I allele II/(ID+DD)=0.048 (table 2). Apparently, the dominant and recessive association of the allele D is greater than that of the allele I (17.904 and 8.958 times, respectively). The results are in agreement with the above literature.

Table 2. The dominant and recessive models revealed alleles on separate groups and at the population level

Groups	Models			
	Dominant		Recessive	
	D	I	D	I
1	8.0	1,5	0.250	0.125
2	-	1	0.333	0
3	99.0	1.029	0.538	0.010
4	11.0	1.2	0.714	0.091
Total at the population level	**20.625**	**1.152**	**0.430**	**0.048**

There are many association studies showing influence of ACE I/D polymorphism on the onset of diabetes mellitus (Al-Saikhan et al., 2017, Pirozzi et al., 2018, Mirfeizi et al., 2018, Aggarwal et al., 2016, Ohkuma et al.,2019). However, recent findings do not support this statement. For instance, several studies done on Japanease and Caucasion population indicate the assosiation of the DD genotype with risk of type 2 diabetes while in Gujarati population (India) this assosiation was not found (Doshi et al., 2015). This result was confirmed in other studies in different ethnic groups, both in patients with and without nephropathy (Arfa et al., 2008, Eroglu et al., 2008, Van-Valkengoed et al., 2008). Thus, the usage of ACE polymorphism as an independent factor responible for diabetes is questionable. However, we can not rule out the other effective genetic factors and environmental factors on the possible role of ACE in the onset of diabetes (Cassis et al., 2019).

Based on the frequency distribution of ACE genotypes showed that ID and II genotypes are frequent in high performance endurance athletes while DD genotype is mainly found among low and middle performance endurance athletes (Hadi et al., 2019). It has been observed that I allele with phenotypes related more to strength than to endurance in 1,027 teenagers (Moran et al. 2006). It suggests a more complicated role for the ACE gene in human physical performance than previously described. Thus, a modest influence of ACE gene on physical performance is clear in general population. Now, the challenge is to clarify the mechanism of ACE influence on performance related phenotypes.

CONCLUSION

We investigated the insertion/deletion (I/D) polymorphism of the angiotensin-converting enzyme (ACE) gene in the Azerbaijani population among 346 people refering to different age groups, nonrelatives and different activities. Based on the results of the PCR method the ressesive and dominant model of both allele was performed. According to the models the D alllele is highly assosciated with the any particular trait than that of the I allele. This finding suggests that the ID polymorphisms of the ACE gene may play an important role susceptibility to schizophrenia in Azerbaijan population. Moreover, the results obtained from the athletes also revealed high susceptibility of ID or DD polymorphism of ACE gene to different sport performances. Additionally, the role of ACE gene polymorphism in the risk of diabetes was researched and ID genotype was mainly found among patients with type 1 and 2 diabetes. However, these findings require repetition in larger samples. Since the features and localization of functional polymorphisms of the ACE gene have not yet been fully understood, our study confirmed that most of them are closely associated with I/D polymorphism. Thus, the physiological significance of ACE polymorphism should be understood as its relationship with the levels of ACE expression in plasma.

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Azərbaycan populyasiyasında angiotensin çevirən fermentin (AÇF) geninin insersiya/delesiya (I/D) polimorfizmi

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Angiotenzin-çevirən ferment renin-angiotenzin-aldosteron sisteminin (RAAS) açar fermentlərindən olub qan təzyiqinin tənzimlənməsində bilavasitə iştirak edir. Güman edilir ki, bu fermenti kodlaşdıran genin (ACE geni) 16-cı intronunda uzunluğu ~287 n.c. olan Alu təkrarların olması/olmaması ilə şərtlənən insersiya/delesiya (I/D) polimorfizmi bəzi xəstəliklərin yaranma riski ilə, o cümlədən ürək-damar xəstəlikləri, müxtəlif tip psixi pozuntular, Alzheymer xəstəliyi, hestasion şəkərli diabetlə və s. assosiasiya təşkil edir. Azərbaycan populyasiyası üçün ACE geninin I/D polimorfizmi üzrə məlumatların olmadığını nəzərə alaraq tərəfimizdən bu genin polimorfizmi spesifik praymer cütlərindən (Hace3s və Hace3as (I), ACE-F və ACE-R (II)) istifadə etməklə PZR metodu ilə tədqiq edilmişdir. Tədqiqatın obyekti 4 qrupa bölünmüş 346 nəfərin qanından ayrılan DNT nümunələri olmuşdur: (1) müxtəlif psixi pozuntuları olan xəstələr (90 nəfər); (2) idmanın müxtəlif növləri ilə məşğul olan gənc idmançılar (84 nəfər tələbə); (3) müxtəlif tipli şəkərli diabetli xəstələr (28 nəfər I tip ŞD (3A subqrupu) və 72 nəfər II tip ŞD (3B subqrupu)); (4) müxtəlif yaşlara və ixtisaslara malik şərti sağlam insanlar (72 nəfər, nəzarət qrupu). Hər iki praymer cütü ilə II genotipli 16 nəfər (4.6%, I-allelə görə homoziqot ko-dominantlar), DD genotipli 101 nəfər (29.2%, D-allelə görə homozqot ko-dominantlar) və ID genotipli 228 nəfər (66.2%, hər iki allelə görə heteroziqotlar) aşkar edilmişdir. Rastgəlmə tezlikləri: f_I=0,373, f_D=0,627, N_D:N_I=1,681. Populyasiya daxilində ayrı-ayrı genotiplərin nisbətləri: ID:DD=2,173; ID:II=11,125; DD:II=6,500. D-allelinə görə dominant modelin (DD+ID)/II=20,625 və resessiv modelin DD/(ID+II)=0,430 qiymətlətinin I allelinə görə dominant (II+ID)/DD=1,152 və resessiv II(ID+DD)=0,048 modellərin qiymətləri müqayisəsi onu göstərir ki, həm dominant, həm də resessiv model üzrə D allelinin hər hansı bir müəyyən əlamətlə (genetik) ilişgisi ehtimalı I allelinə nisbətən daha yüksəkdir (uyğun olaraq 17.904 və 8.958 dəfə). Bu nəticələr bir çox patologiyalar və ya xəstəliklərlə məhz D allelinin assosiasiya təşkil etməsi haqqında ədəbiyyat məlumatlarını təsdiq edir. Alınan nəticələrin analizi həm qruplar daxilində, həm də qruplar arasında tədqiq olunan əlamətlərin D allelindən asılılığının statistik etibarlı şəkildə ($P \leq 0.01$) korrelyasiya etdiyini aşkar etmişdir.

Açar sözlər: *Renin-angiotensin-aldosteron sistemi (RAAS), angiotenzin-çevirən ferment (AÇE), insersiya, delesiya, hipertenziya, psixi pozuntular, şəkərli diabet (ŞD), polimorphizm, ko-dominant, homozigot, heteroziqot, COVID-19*

Инсерционный/делеционный (I/D) полиморфизм гена ангиотензин-превращающего фермента (АПФ) в Азербайджанской популяции

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Ангиотензин-превращающий фермент (АПФ) является ключевым ферментом ренин-ангиотензин-альдостероновой системы (РААС), которая непосредственно участвует в регуляции артериального давления. Предполагается, что инсерционный/делеционный (I/D) полиморфизм гена этого фермента (гена ACE), благодаря наличия/отсутствия в 16-м интроне Alu-повторов длиной ~287 п.н., ассоциирован с риском развития некоторых заболеваний, включая сердечно-сосудистые заболевания, различные виды психических расстройств, болезнь Альцгеймера, гестационный диабет и др. Учитывая отсутствие данных по I/D-полиморфизму гена ACE для Азербайджанской популяции мы

изучали полиморфизм этого гена методом ПЦР с использованием конкретных пар праймеров (Hace3s и Hace3as (I), ACE-F и ACE-R (II)). Объектом исследования служили образцы ДНК, выделенные у 346 человек, разделенных на 4 группы: (1) пациенты с различными психическими расстройствами (90 пациентов); (2) группа юных студентов, занимающихся различными видами спорта (84 человека мужского пола); (3) больные сахарным диабетом (28 пациентов с СД I типа (подгруппа 3A) и 72 пациента с СД II типа (подгруппа 3B); (4) группа условно здоровых людей разного возраста и специальностей (72 человека, контроль). Обе пары праймеров идентифицировали 16 лиц с генотипом II (4,6%, гомозиготные ко-доминанты по I-аллелю), 101 человек с генотипом DD (29,2%, гомозиготные ко-доминанты по аллелю D) и 228 лиц с генотипом ID (66,2%, гетерозиготы по обоим аллелям). Частота встречаемости составила: f_I=0,373, f_D=0,627, N_D:N_I=1,681. Соотношение отдельных генотипов в исследуемой популяции: ID:DD=2,173; ID:II=14,125; DD:II=6.500. Сравнение значений доминантной модели для аллеля D - (DD+ID)/II=20,625 и рецессивной модели DD/(ID+II)=0,430 относительно доминантной модели для аллеля I – (II+ID)/DD=1,152 и рецессивной модели для аллеля I – II/(ID+DD)=0,048 указывает на то, что как в доминантной, так и в рецессивной моделях вероятность ассоциации аллеля D с конкретным признаком выше, чем у аллеля I (в 17,904 и 8,958 раза соответственно). Эти результаты подтверждают литературные данные об ассоциации именно аллеля D со многими патологиями или заболеваниями. Анализ полученных данных также выявил достоверную корреляцию (P≤0,01) исследуемых признаков аллеля D как внутри групп, так и между группами.

Ключевые слова: *Ренин-ангиотензин-альдостероновая система (РААС), ангиотензин-превращающий фермент (АПФ); инсерция, делеция; гипертония, психические расстройства, сахарный диабет (СД), полиморфизм, кодоминантный, гомозиготный, гетерозиготный, COVID-19*

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Overview on under development of vaccine candidates against SARS-CoV-2

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Severe acute respiratory syndrome SARS -CoV-2 is a newly emerging infectious disease caused by a novel coronavirus, SARS-CoV. The World Health Organization announced the outbreak of coronavirus disease (COVID-19) pandemic on 11 March 2020. SARS-CoV-2 and the Middle Eastern respiratory syndrome-related coronavirus (MERS-CoV) constitute the most life-threatening species among all human coronaviruses. Until now, not any vaccines have been developed against coronaviruses. Therefore, it is essential to develop vaccines to prevent outbreaks of COVID-19. Live attenuated, inactivated, subunit, recombinant protein, epitope, DNA, RNA based vaccines, adenovirus-based vectors, virus-like particle vaccine forms the bases of vaccine candidates against COVID-19. Each current vaccine strategy has distinct advantages and disadvantages. Therefore, it is paramount that multiple strategies be advanced quickly and then evaluated for safety and efficacy. According to the World Health Organization report, 42 COVID-19 vaccine projects are in clinical evaluation. Vaccine candidates developed against COVID-19 are different from the vaccine candidates previously developed against SARS-CoV, MERS-CoV, and have a wider platform and are new hopes to develop a vaccine against COVID-19.

Keywords: *SARS-CoV-2, COVID-19, vaccines*

INTRODUCTION

Severe acute respiratory syndrome SARS -CoV-2 is a newly emerging infectious disease caused by a novel coronavirus, SARS-CoV. The World Health Organization announced the outbreak of coronavirus disease (COVID-19) as a pandemic on 11 March 2020. WHO reported that ~80% of COVID-19 patients have mild-to-moderate symptoms, while ~20% develop serious manifestations such as severe pneumonia, acute respiratory distress syndrome (ARDS), sepsis, and even death. The virus SARS-CoV-2 belongs to the genus Betacoronavirus (β-CoV) of the family Coronavirdae SARS-CoV-2 have a single-stranded positive sense RNA genome, Encoding 4 structural proteins; spike (S), envelope (E), membrane (M), and nucleocapsid (N) which S is a major protective antigen that elicits highly potent neutralizing antibodies (NAbs), 16 non-structural proteins (nsp1-nsp16) and several accessory proteins (Rabaan et al., 2020). The SARS-CoV spike (S) protein is composed of two subunits; The S1 subunit contains a receptor-binding domain that engages with the host cell receptor angiotensin-converting enzyme 2 (ACE2). The S2 subunit mediates fusion between the viral and host cell membranes. The S protein plays key parts in the induction of neutralizing-antibody and T-cell responses, as well as protective immunity, during infection with SARS-CoV. The vaccine is a non-pathogenic immunobiological substance that has specific protection against any disease. The immunobiological substance causing

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recognition and elimination of the foreign agent by stimulates the body's immune system cells. The immunobiological substance should consist of particles of microbial agents or synthetic forms of these particles. The ideal vaccine Should be Immune effective and cause long time immunity, Can be stored for a long time, Immunity should be sufficient with a single dose, Should not have any side and toxic effects, Should be cost effective and easy to find, Must be reliable Should not cause infection. The mRNA-based vaccine targeted to the S protein of SARS-CoV-2 works by active immunization (Rabaan et al., 2020; Chen et al., 2020). This technique will not use part of the virus but only recombine mRNA of the S protein in vitro according to the gene sequence, which is coated with lipid nanoparticles for effective delivery. Once injected into the muscle, the myocytes take up the lipid nanoparticle (LNPs) and then release the mRNAs into the cytoplasm for translation into the S proteins. These endogenously synthesized S proteins will be secreted to activate both humoral and cellular immune responses. S protein – spike protein; IM – intramuscular, LNP – lipid nanoparticle; DC – dendritic cell; MHC – major, histocompatibility complex; Ag – antigen. In one study researchers used S377–588-Fc protein as a pattern antigen and evaluated the effects of different adjuvants on the stimulation of host immune responses to MERS coronavirus recombinant binding domain (RBD) based subunit vaccine. They used dissimilarly formulated vaccines to immunize mice and then gave a demonstration of the comparison of MERS coronavirus typical humoral immune responses and neutralizing antibodies, as well as T cell-mediated immune responses (Zhang et al., 2016). No any specific drugs or vaccines have developed for the treatment and prevention of COVID-19 till now. This article aims to share information about the recent potential vaccine candidates against SARS-CoV-2.

Vaccine development against SARS-CoV-2

Various types of vaccines such as DNA-, RNA-based formulations, recombinant-subunits containing viral epitopes, adenovirus-based vectors, and purified inactivated virus are under development against SARS-CoV-2. Traditionally vaccine development methods such as Purified inactivated viruses have been found to be effective safe against viruses like influenza and poliovirus.

Inactivated vaccines

Inactivated virus vaccines are obtained by killing viruses by various methods. The methods used in the virus inactivation are essentially preserved antigenic properties by disrupting the reproductive abilities of the virus. It mostly stimulates the humoral immune response. Inactivated vaccines must be noninfective, biosafe. Methods used in inactivation vaccines include Heat, UV, Formaldehyde (exm HAV), Beta prophylactone (influenza, jerusalem), Phenol (Chua et al., 2018). In one study scientists developed a pilot-scale production of a purified inactivated SARS-CoV-2 virus vaccine candidate (PiCoVacc), which induced SARS-CoV-2-specific neutralizing antibodies in mice, rats, and non-human primates. These antibodies neutralized 10 representative SARS-CoV-2 strains, suggesting a possible broader neutralizing ability against SARS-CoV-2 strains. These data support the clinical development of SARS-CoV-2 vaccines for humans (Gao et al., 2020). Thus, the obtained results with the inactivated vaccine candidate against SARS-CoV and SARS-CoV-2 reveal that this approach is promising in developing the vaccine against both types of coronavirus.

Live attenuated vaccines

Live vaccines are vaccines which virulence is reduced or eliminated but are prepared without degrading their antigenic nature. Live vaccines create both humoral and cellular immunity and form a large number of memory cells. Immunization of live vaccines usually lasts a lifetime. Live-attenuated vaccines reveal high immune responses that can present to long term immunity after first or second time vaccination. These vaccines have been used against measles, yellow fever, chickenpox, infections, and several other diseases. This type of vaccine usually is comparatively easy to generate for viruses, but it is not easy to create for bacteria and parasites (Minor, 2015). An ideal vaccine candidate has to contain genetic selectors that could be separately attenuating and also could be stimulated by recombination processes. In one study researchers inoculated the mice with UV-inactivated SARS-CoV in the presence or absence of adjuvant. After vaccination, a high rate of humoral immune response that revealing to the production of long-term antibody expression and the memory B cells was recognized. Antibodies which was generated

in mice against SARS-CoV observed both S (spike) and N (nucleocapsid) proteins of the pathogen and could prevent the infection (Takasuka et al., 2004). Thus, the results obtained with the inactivated vaccine prepared against SARS-CoV reveal that this approach is important in developing the vaccine against SARS-CoV-2.

Subunit vaccines

A number of technologies that target S protein have been previously used for the development of vaccines and antiviral therapeutics. The generation of subunit vaccines appropriates to ensure wide prevention should target the variety of the major immunogenic agents of the Spike (S) protein (Malik et al., 2020). Subunit vaccines for both SARS coronaviruses rely on eliciting an immune response against the S-spike protein to prevent its docking with the host ACE2 receptor. scientists have developed and tested a subunit vaccine comprised of only the receptor-binding domain (RBD) of the SARS-CoV S-protein when formulated on alum, the SARS-CoV RBD vaccine elicits high levels of protective immunity on the homologous virus challenge. An advantage of the RBD-based vaccine is its ability to minimize host immunopotentiation (Wang et al., 2020). Initial findings that the SARS-CoV and SARS-CoV-2 RBDs exhibit more than 80% amino acid similarity and bind to the same ACE2 receptor offer an opportunity to develop either protein as a subunit vaccine. Thus, subunit vaccines do not include the whole pathogen, but only the specific compounds or antigens which induce the immune system.

Recombinant protein vaccines

A recombinant vaccine is a vaccine produced by using recombinant DNA technology. In this technology, certain protein antigens can be produced in bacteria, yeast, mammalian cells, or plants. Recombinant protein vaccines or recombinant subunit vaccines compared to other vaccine platforms have the best biosafety property, such as inactivated virus, live-attenuated virus, and viral vector-based subunit vaccines. Recombinant protein-based vaccines do not rise a risk for incomplete, inactivation, recovery of virulence of the attenuated virus, or undesirable responses of host cells to virus vectors. In one study researchers synthesized a recombinant adenovirus type-5 (Ad5) vectored COVID-19 vaccine expressing the S glycoprotein of a severe acute respiratory syndrome (SARS-CoV-2) strain. After an experiment on 18-60 years' adults, they demonstrated that the Ad5 vectored COVID-19 vaccine is tolerable and immunogenic at 28 days' post-vaccination. Humoral responses against SARS-CoV-2 peaked at day 28 post-vaccination in healthy adults, and rapid specific T-cell responses were noted from day 14 post-vaccination. The results suggest that the Ad5 vectored COVID-19 vaccine warrants further investigation (Zhu et al., 2020).

Preparation of plant based recombinant protein vaccines

Recombinant proteins based on plants are safe, effective, and inexpensive. In one study researchers showed that SARS-coronavirus (CoV) spike protein (S protein) and its truncated fragments have expressed (the N-terminal) in tomato and low-nicotine tobacco plants. Incorporation of the S1 fragment into plant genomes as well as its transcription was confirmed by PCR and RT-PCR analyses. High levels of expression of recombinant S1 protein were observed in several transgenic lines by Western blot analysis using specific antibodies. Plant-derived antigen was evaluated to induce systemic and mucosal immune responses in mice. Mice showed significantly increased levels of SARS-CoV-specific IgA after oral ingestion of tomato fruits expressing S1 protein. Sera of mice parenterally primed with tobacco-derived S1 protein revealed the presence of SARS-CoV-specific IgG as detected by Western blot and ELISA analysis

Epitope vaccines

Epitopes are the part of antigens that identify by the immune system, specifically by, B and T cells antibodies. in recent years new approaches in vaccine technology based on epitopes show great immunity both in humans and pathogens. Epitope-based vaccines can be consist of the short peptide with poor immunogenicity or longer peptides composed of multiple epitopes, based on dendrimer structures such as multiple-antigenic peptides (Palatnik-de-Sousa et al., 2018). In one study researchers demonstrated the potency of epitopes from the S protein of MERS-CoV and detected that

the antigenic epitopes may present as effective vaccines for the prevention of MERS-COV pathogen (Tahir Ul Qamar et al., 2019).

In another study researcher to obtain immunogenic epitopes, characterized spike glycoprotein. They choose 13 Major Histocompatibility Complex-(MHC) I and 3 MHC-II epitopes, having antigenic properties. To increase fast immunogenic property of these epitopes they performed immunoinformatics analysis. Moreover, they demonstrated that the molecular docking of vaccine components with the TLR-5 proves the significance and effectiveness of these epitopes as an ideal vaccine candidate against SARS-COV-2. Development of epitope-based peptide vaccine against novel coronavirus 2019 (SARS-COV-2) (Bhattacharya et al., 2020). in one study scientists designed a multi-peptide subunit-based epitope vaccine against COVID-19. The recombinant vaccine contains cytotoxic T-lymphocyte, an adjuvant, T-lymphocyte, and B-cell epitopes joined by linkers. The computational data demonstrate that the vaccine is non-toxic, non-allergenic, thermostable, with the capability to elicit a humoral and cell-mediated immune response. The stabilization of the vaccine construct is validated with molecular dynamics simulation studies. This unique vaccine consists of 33 highly antigenic epitopes derived from three proteins and plays an important role in viral entry and pathogenicity and host-receptor recognition. They recommend that this vaccine be synthesized and tested quickly (Kalita et al., 2020).

Virus-like particle vaccines

Compared to other traditional live virus vaccines, virus-like particles are more biosafe and effective. VLP based vaccines are not infective. As VLPs don't require inactivation or attenuation it makes this technology so important. If special natural proteins, that have the ability to suppress immune response, eliminated from the VLP structure can remarkably improve the potential of these particles. Virus-like particles, obtained by different expression methods, have been broadly used as vaccines and delivery systems for drugs and genes (Charlton Hume et al., 2019). VLPs have extremely repetitive surface they are capable to boost the high level of B-cell responses in case using adjuvant.

Bacterium like particle vaccines

The action of Bacterium-like particles based on the membrane surface receptor TLR2 (innate receptor) activation which is specific for different viral, fungal, and bacterial compounds. The BLPs are new types of immunostimulators (Van Braeckel-Budimir et al., 2013). To increase protective immunity BLPs can be used in combination with antigens. The successful attaches of antigen to particle could boost the immune response. BLPs have been successfully used for Streptococcus pneumonia, influenza, and Yersinia pestis (Nganou-Makamdop et al., 2012; Saluja et al., 2010). researchers designed a bacterium-like particle (BLP) vaccine against MERS-CoV, presenting the recombinant binding domain (RBD) antigen protein. The results of this research indicated that BLP based vaccine can stimulate a high characteristic mucosal immune response. They suggested that MERS-CoV bacterium-like particle with GEL01 adjuvant is a potential platform for vaccine development (Li et al., 2019).

Polymeric nanoparticle based vaccines

Nanoparticle-based vaccines have gained more importance in recent years because of their more effectiveness, offering numerous advantages over inactivated or subunit vaccines, the ways of immunization, and features such as boosting the immune response as a targeted carrier system. Nano particle-based vaccines could be produced by encapsulating vaccine compounds within nanoscale particles or by binding viral antigens to the surface of the particle. These nanoparticles can prevent the degradation effect of proteolytic enzymes on antigens along with their availability and preserve the prolonged and systematic release of antigens. These features of nanoparticle vaccines give the opportunity to stimulate high immune responses in contrast to soluble antigen vaccines (Dhakal & Renukaradhya, 2019). This technology also is considered a new approach to generating vaccines against MERS-CoV, RSV, and Epstein Barr virus.

Inorganic nanoparticle based vaccines

Physicochemical features of inorganic nanoparticles make it a suitable option for use in immunotherapy applications because these specifications prohibit the generation of antibodies against

the platform. In addition, some studies demonstrate that different immune cells, such as macrophages, dendritic cells, and lymphocytes, that are induced via Gold nanoparticles and AuNPs, cause the generation of pro-inflammatory and Th1 cytokines. One study, explored the effectiveness of AuNPs and TLR agonists. Of these two kinds of vaccine adjuvants, AuNPs are used as antigen delivery systems and adjuvants for subunit vaccines. The antibodies that were stimulated by recombinant S (spike) protein prevented SARS-CoV infection, but an eosinophilic immunopathology was recognized in the lungs of immunized mice after SARS infection. According to this study, an adjuvant is necessary for the prevention of eosinophilic immunopathology in the lungs after SARS-CoV infection, even with the spike (S) protein vaccine. Researchers in one study designed a recombinant spike (S) protein of SARS coronavirus by using the expression system of baculovirus. Furthermore, they investigated the effectiveness of the vaccine and its ability to stimulate lung eosinophilic immunopathology in the murine SARS model (Sekimukai et al., 2019).

DNA vaccines

DNA vaccine technology is a new effective way to induce humoral and cellular immune responses to protein antigens. Bacterial plasmids are the DNA vaccines component. antigen expression unit and production unit include bacterial sequences are expression plasmids which use in DNA-based vaccination technology. DNA vaccines have been used against several diseases (such as influenza, HIV, Ebola, West Nile and other viruses) in various animal models (Gurunathan et al., 2000). In contrast to success in animal models, the use of these vaccines on humans have been carried out just in recent years. More investigations are needed to demonstrate its potential to prevent human diseases. According to the disadvantages of the animal model for SARS-CoV pathogen, to determine the immunogenicity of plasmids in humans is important. The description of efficacious virus genes can lead to the option of inserts for vaccination technologies that based on genes According to one study, DNA vaccination has been used to stimulate cellular and humoral immunity against S glycoprotein of SARS-CoV. The humoral immunity that involves the expression of NA (neutralizing antibodies) can prevent the replication of viruses in the experimental animal model and recommend that this kind of vaccination reveals prophylactic immune response (Yang et al., 2004). In one study, researchers explored the biosafety, immunogenicity, and endurance of MERS coronavirus DNA-based vaccine (GLS-5300) They demonstrated that this DNA vaccine candidate did not reveal undesirable side effects and stimulated identical cellular and humoral responses. Immune responses were recognized in most of the persons (Σ85%) that participate in trials after vaccination twice and lasted through 1 year. They noted that this phase-1 clinical research does not evaluate the effectiveness of DNA vaccine and it needs further investigations in an endemic region of MERS-CoV (Modjarrad et al., 2019).

Messenger RNA vaccines

RNA or mRNA is a new vaccine technology that provides immunity through RNA containing the vector. mRNA vaccines have shown strong immunity against infectious disease, influenza virus, Zika virus, and others. The absence of genome integration, the production of multimeric antigens, rapid development capacity, the stimulation of immune response against different diseases, make mRNA vaccines more effective than traditional vaccine candidates. mRNA vaccine development technology involves the capability to combine several mRNAs into a single vaccine and to induce more potent protective immunity. Messenger RNA vaccine development technology involves the capability to combine several mRNAs into a single vaccine and to induce more potent protective immunity. However, some limitations also were evaluated, such as instability of messenger RNA, problems with carriage of mRNA into cells and etc. For this, the design of biosafe and effective products for *in vivo* carriage of mRNA and improved procedures to produce high-grade mRNA are required. Previously, scientists generated mRNA vaccine candidate that prevents Zika virus in mice and monkeys after injection of a single dose. Researchers were used mRNA vaccines produced for protection from influenza viruses (A H1N1, H3N2 and H5N1) vaccine prompts B and T cell-dependent protection. It purposes several antigens, containing strongly protected virus nucleoprotein, displaying

its profit as a cross-protective vaccine candida. Recombinant vector vaccines caused both cellular and humoral immunity, adenovirus vectored vaccines are one of the widely used recombinant vector vaccines against several diseases including HIV, influenza, malaria etc. According to previous animal researches, different vaccine candidates showed effectiveness, biosafety, and immunogenicity against MERS-CoV. Among these options, the recombinant viral vector-based candidates are evaluated as the most suitable platform (Wang et al., 2020; Pardi et al., 2017).

Recombinant vector vaccines

A vector vaccine is obtained by genetic engineering. Vector vaccines prepare from live microorganisms which are non-pathogenic or have low pathogenicity for the desired species and induce an immune response against micro-organisms by encoding antigens via genes. viral vectors are a tool used to deliver genetic material into cells. This process can be done in a living organism (*in vivo*) or in cell culture (*in vitro*).

In one study researchers synthesized AOaV-1 based topical respiratory vaccine candidate against CoVID-19. They engineered a virulent strain of AOaV-1 to express full-length spike (S) glycoprotein which is highly neutralizing and is a major protective antigen of the SARS-CoV-2. As a result, they mentioned that the recombinant vaccine vector stably expressed S protein after multiple propagations in chicken embryonated eggs, and this expression did not remarkably affect the in vitro growth characteristics of the recombinant. And they inform that the synthesized vaccine carries the potential for clinical studies against COVID19 (Rohaim & Munir, 2020).

CONCLUSION

According to the World Health Organization (WHO) report, 42 COVID-19 vaccine projects are in clinical evaluation. Vaccine candidates developed against COVID-19 are different from the vaccine candidates previously developed against SARS-CoV, MERS-CoV, and have a wider platform and are new hopes to developing of a vaccine against COVID-19. Until now, no vaccines have been applied against coronaviruses. Therefore, it is essential to develop vaccines to prevent outbreaks of COVID-19. Live attenuated, inactivated, subunit, recombinant protein, epitope, DNA, RNA based vaccines, adenovirus-based vectors, virus-like particle vaccine forms the bases of vaccine candıdates against COVID-19.

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SARS-CoV-2-ə qarşı hazırlanmaqda olan peyvənd namizədlərinə ümumi baxış

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Ağır kəskin tənəffüs sindromu koronavirus 2 (SARS-CoV-2) yeni bir koronavirüs olan SARS-CoV'un sebep olduğu yeni bir yoluxucu xəstəlikdir. Ümumdünya Səhiyyə Təşkilatı 11 mart 2020-ci il tarixində koronavirus xəstəliyinin (COVID-19) yayılaraq pandemiyaya səbəb olduğunu elan etdi. SARS-CoV-2 və Yaxın Şərq tənəffüs sindromuna (MERS-CoV) səbəb olan koronavirus insanlar üçün ən təhlükəli koronavirus növlərdir. İndiyə qədər koronaviruslara qarşı hər hansı bir peyvənd hazırlamaq mümkün olmamışdır. Bu ədəbiyyat icmalında COVID-19-a qarşı dünyanın müxtəlif laboratoriyalarında hazırlanan peyvəndlər haqqında məlumat verilməklə birlikdə onların müsbət və mənfi tərəfləri müzakirə edilmişdir. Hazırda COVID-19-a qarşı canlı, zəifləmiş, təsirsiz hala gətirilmiş, subunit, rekombinant zülal, epitop, DNT və RNT əsaslı vaksinlər, adenovirus əsaslı vektorlar, virusa bənzər hissəciklərin əsasında namizəd peyvəndlər üzərində tədqiqatlar aparılmaqdadır. Ümumdünya Səhiyyə Təşkilatının hesabatına görə 42 COVID-19_a qarşı peyvənd layihəsi klinik qiymətləndirmədədir. Hazırlanacaq peyvəndlərin qiymətləndirilməsində onların biotəhlükəsizliyinə və effektivliyinə diqqət emək çox vacibdir. COVID-19-a qarşı hazırlanan peyvənd namizədləri, əvvəllər SARS-CoV, MERS-CoV-a qarşı hazırlanan peyvənd namizədlərindən fərqli olub, daha geniş bir platformaya sahibdir. Buna görə də aparılan bu tədqiqatlar COVID-19-a qarşı peyvənd işlənilib hazırlanmasında yeni ümidlər verir.

Açar sözlər: *SARS-CoV-2, COVID-19, vaksinlər*

Обзор разрабатываемых вакцин-кандидатов против SARS-CoV-2

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Тяжелый острый респираторный синдром - коронавирус 2 (SARS-CoV-2) - это недавно возникшее инфекционное заболевание, вызывающее коронавирусную болезнь (COVID-19). Всемирная организация здравоохранения объявила о пандемии коронавирусного заболевания (COVID-19) 11 марта 2020 года. SARS-CoV-2 и коронавирус, связанный с ближневосточным респираторным синдромом (MERS-CoV), считаются наиболее опасными коронавирусами для жизни человека. До сих пор не было разработано вакцин против коронавирусов. Следовательно, для предотвращения вспышек COVID-19, очень важно разработать основы вакцин-кандидатов против COVID-19: аттенуированные, инактивированные, субъединичные вакцины, вакцины из рекомбинантного белка, эпитопа, вакцины на основе ДНК и РНК молекул, вакцины на основе векторов-аденовирусов, вакцины на основе вирусоподобных частиц. Каждая текущая стратегия вакцинации имеет определенные преимущества и недостатки. Поэтому крайне важно быстро разработать несколько стратегий и затем оценить их безопасность и эффективность. Согласно отчету Всемирной Организации Здравоохранения 42 проекта вакцин против COVID-19 проходят клиническую оценку. Вакцины-кандидаты, разработанные против COVID-19, отличаются от вакцин-кандидатов, ранее разработанных против SARS-CoV, MERS-CoV, имеют более широкую платформу и являются новой надеждой на разработку вакцины против COVID-19.

Ключевые слова*: *SARS-CoV-2, COVID-19, вакцины

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Rapid and simple detection of SARS-CoV-2 with point-of-care COVID-19 testing

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COVID-19 as a public health concern of the world has spread worldwide and a combination of various methods including tomography imaging, reverse transcription-polymerase chain reaction (RT-PCR), enzyme-linked immunosorbent assay (ELISA) and cell culturing were developed to detect and identify SARS-CoV-2. Due to the absence of specific antiviral agents or vaccines for COVID-19 treatment, early detection and identification are vital. An alternative, sensitive, fast point-of-care (POC) detection tool that can be routinely used by health care providers utilizing biological fluids as a specimen before starting an emergency process is desired. Efforts are underway to develop more effective diagnostic and surveillance technologies with loop-mediated isothermal amplification (LAMP) tests, antibody testing and microfluidic RT-PCR devices (Lab-on-a-chip). Point-of-care diagnostics are promising candidates in SARS-Cov-2 detection and encourage scientists to improve their technologies beyond conception. The reverse transcription LAMP (RT-LAMP) method developed for SARS-CoV-2 could detect the virus even in saliva samples in less than an hour (Harapan et al., 2020). Lab-on-a-chip devices contain a small size chip, microchannel, microelectrodes and microheater. Cell lysis, DNA extraction and PCR amplification stages could be integrated on these microchips (Sharma et al., 2020). Because of the rapid detection, small volume of the specimen and integration with PCR in a portable tiny system, these devices are promising for SARS-CoV-2 detection (Huang et al., 2018). The validity and sensitivity of all the above-mentioned methods need to be improved for salivary specimen usage; in case of improvement, they might provide an opportunity for salivary detection of the virus without a waiting period and complex analytical infrastructure.

Keywords: *COVID-19; RT-PCR; polymer chip; thermocycler; emergency situations; biosensors*

INTRODUCTION

The family of coronaviride are responsible for respiratory, hepatic, neurological and enteric manifestations of the new epidemy of COVID-19. *Coronaviride* family has four general classes including, alpha, beta, gamma and delta coronaviruses (Harapan et al., 2020). The crown-like shape of the virus under electron microscope has been attributed to the term coronom (Sharma et al., 2020). A broad range of mammels such as humans, animals and rodents could be infected via coronaviruses. High recombination and mutation rate leads to the rapid adjusting of the virus in the new host (Sharma et al., 2020; Huang et al., 2018).The common symptoms of this pandemic health concern include fever, cough, myalgia, plummeted leukocytes and opacities (Chan et al., 2020; Hoffmanet al., 2020). Headaches, abdominal pain, hem-

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optysis and diarrhea are considered as other symptoms (Harapan et al., 2020).However, many cases are asymptomatic and the validity of testing assays is low, and thus the real number of affected patients is certainly underestimated. This in turn increases the spreading of the disease (To et al., 2020). In addition, the symptoms are commonly similar to normal cold and flu. So, accurate and early diagnosis of the disease is crucial in order to prohibit the fatality. In particular, early detection allows the clinicians to avoid serious complications in COVID-19 patients. Also, identification of negative individuals helps the removal of unnecessary quarantine time. In the current paper, novel developments in the diagnosis of COVID-19 and innovations especially in the context of biosensors and point of care settings will be discussed.

MATERIAL AND METHODS

1. Rapid and portable detection devices

The nonspecific manifestations of COVID-19 require an urgent need for rapid diagnosis of suspected individuals and an exact screening (Yang et al.,2020). Point of care settings which are rapid, efficient, cost-effective and do not need experts are demanded (Nguyen et al., 2018). These devices could be utilized in emergency situations (Baroneet al., 2020).This would inhibit rapid spreading of the diseases (Nguyen et al., 2020). However, the current standard methodology is based on the real-time reverse transcription-polymerase chain reaction (rRT-PCR) with five steps (Berry et al., 2016) that include sample collection, storing the specimens, transmission to the laboratory, testing protocol and result reporting. In addition, sample transportation to the rRT-PCR central laboratory is time-consuming and thus postpones obtaining the results (Berry et al., 2016; Chu et al., 2020). This is a pivotal drawback in the COVID-19 outbreak since can lead to the rapid distribution of the disease. The need for technical expertise and being very expensive are other problems (Liaw et al., 2012; Cho et al., 2014). Also, co-detection of coronaviruses with other respiratory viruses via PCR might result in false positive cases (Fig. 1) (Cho et al., 2014).

2. Loop-Mediated Isothermal Amplification (LAMP) Assays in PoC devices

Surrogate molecular amplifications methods to overcome the limitations of RT-PCR technique are required. A novel nucleic acid amplification method with high efficiency and specificity under isothermal situations is loop-mediated isothermal amplification (LAMP) (Fig. 2). A DNA polymerase with strand displacement function and a series of four primers are used in the LAMP to fabricate several DNA copies in a short time that are stem-loop with multiple inverted repeats of the target and cauliflower-like shape (Nagamine et al., 2002). In addition to high sensitivity and specificity, simple protocol has turned it into an appealing method in the realm of molecular biology and pathogen detection. Instead of heat denaturation, LAMP used strand-displacement polymerases to synthetize a single-stranded template. The main advantage is diminishing the use of energy in thermocycler because it runs in a constant temperature. In comparison with PCR, this technology is more stable and sensitive (Labarrere et al., 2011; Galvez et al., 2020). So, there is a great hope that LAMP could be a potential candidate in point-of-care detection of COVID-19. In the project of Veterinary validation of point-of-care diagnostic instrument), LAMP has been utilized as a point-of-care device in detection of some respiratory viruses such as avian influenza virus (Galvez et al., 2020). In this project the detection time was then 60 min. also, combination of LAMP with disposable polymer chips as a lateral flow strip would be beneficial. The example for this method might be COVID-19 IgM/IgG Rapid Test of BioMedomics (Cassaniti et al., 2020) with the sensitivity of approximately 89%.

3. New technologies for SARS-CoV-2 detection

Biosensors are novel detection tools which mix the selectivity properties of a biomolecule with the sensitivity of a transducer (Kurbanoglu, 2020). Biosensors can achieve rapid, reliable, real time and sensitive diagnosis of different diseases (Sin et al., 2014). Different types of biosensors have formerly been applied in the detection of infectious diseases and pathogens (Fig. 3) (Sin et al., 2014).

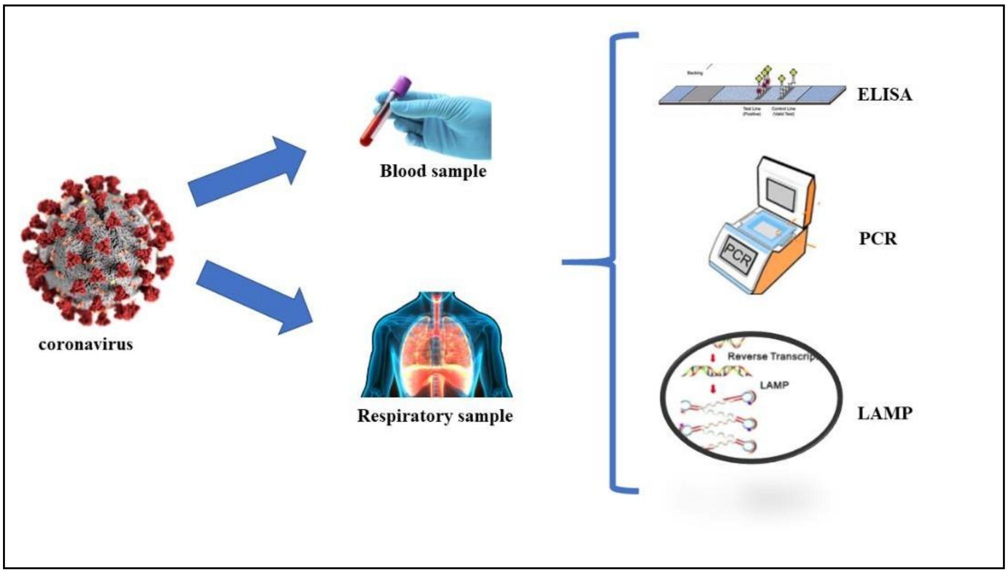


Fig. 1. Current diagnostic methods for SARS-CoV-2 detection from respiratory and serum samples.

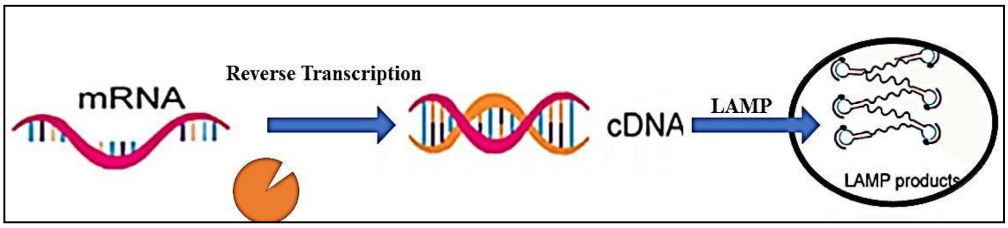


Fig. 2. Illustration of -LAMP amplification method.

The trapping of conductive nanoparticles with smaller wavelength of light is the basis of an optical sensor termed as Localized surface plasmon resonance (LSPR). Coherent localized plasmon oscillation is generated by the interaction of the incidence light and surface electrons in the conduction band. Local alterations such as the differences in the refractive indices and molecular binding change the resonance frequency (Petryayeva and Krull, 2011). Viral sequences such as ORF1ab COVID, RdRp-COVID and SARS-CoV-2 e genes havebeen detected via localized surface plasmon resonance (LSPR) sensing transduction and Dual-functional plasmonic biosensor utilizing plasmonic photothermal (PPT) impact. The in situ hybridization of RdRp of SAR S–CoV-2 and its complementary DNA has been increased using a converted PPT heat energy near the gold nano-islands. Photodermal increased LSPR produced a higher slope compared to the system destitute of phototermal effect. This biosensor could distinguish between SARS-CoV and SARS- CoV-2 viruses. However, a false positive response signal was achieved for RdRp-SARS sequence in the absence of photothermal effect. The detection limit for this fabricated device was estimated to be 0.22 pM (Qiu et al., 2020).

The basis for the field effect transistor (FET) is the regulation of carrier mobility through a biased semiconductor owed to the electrostatic field (Fig. 4). The selective diagnosis of special targets is provided with the covering of the gate surface which is covered with a layer that could be altered using different biomolecules (Ahmad et al., 2020).

The SARS-CoV-2 spike protein S1 was detected using a graphene FET modulated with an antibody of SARS-CoV-2 spike S1 subunit protein (CSAb) or angiotensin-converting enzyme 2 (ACE2).

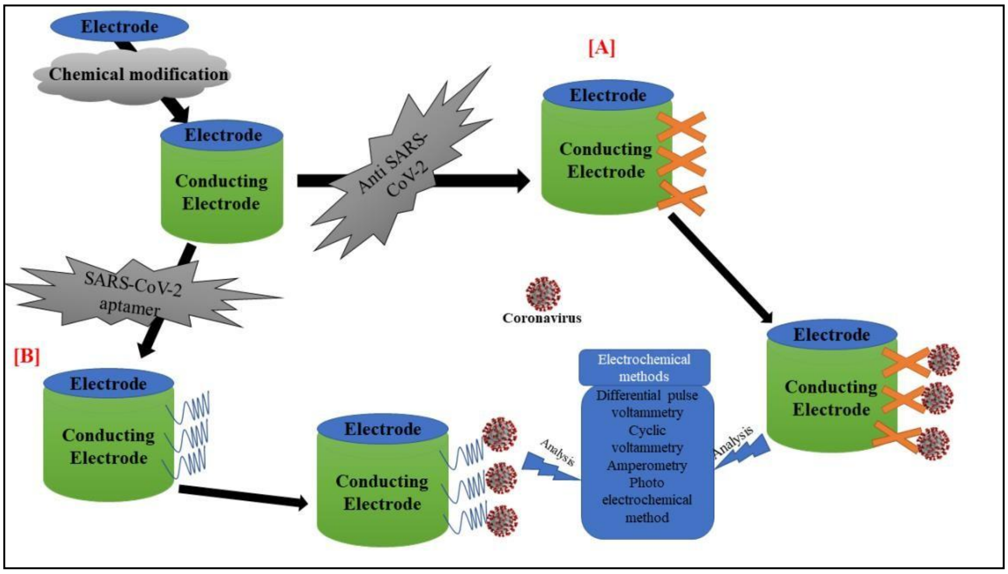


Fig. 3. Electrochemical SARS-CoV-2 sensor. Detection of COVID-19 using specific transducer system (SARS-CoV-2 specific antibodies [A] and Aptamers [B])

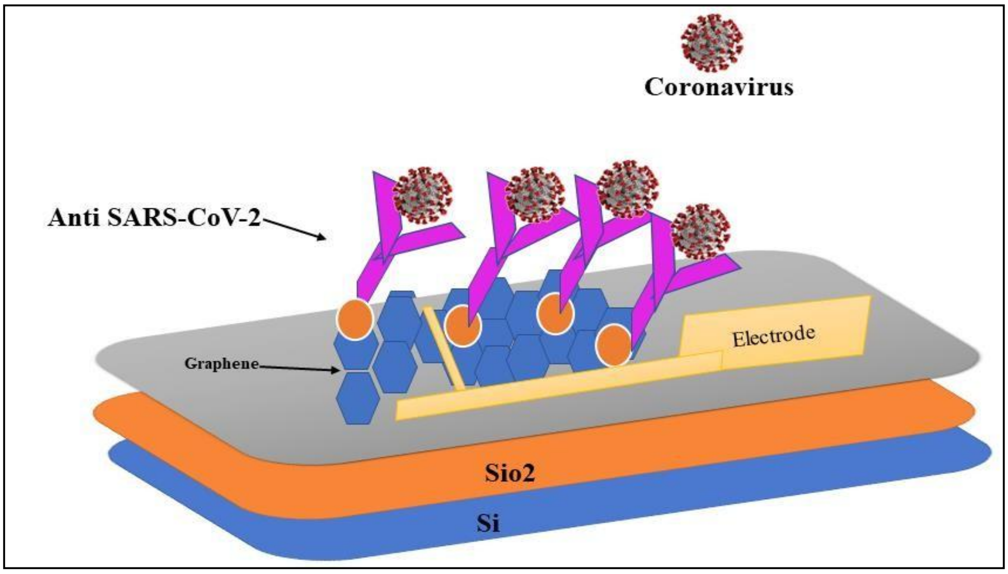


Fig. 4. Field-effect transistor biosensor

The graphen surface encompasses CDAb/ACE2 receptors and the binding of S1 protein with a positive charge mediate the conduction and resistance in the FET. In this study, the CSAb-altered graphene FET showed higher affinity to the antibody and thus a higher sensitivity with an LOD of 0.2 pM (Yüceet al., 2020).

The alterations in channel surface potential of the FET and its impact on the electrical impulse has provided the platform for detection of SARS-CoV-2. The S protein as the main transmembrane protein in the virus and acts as a great antigen but exhibits diversities in the amino acid sequences in coronaviruses.

The SARS-CoV-2 S1 antigen was also detected with a Cell-based potentiometric biosensor A membrane-engineered renal cell changed with

the SARS-CoV-2 SpikeS1 antibody. The antigen/antibody interaction changes the potential of the membrane. Eight gold screen printed electrodes along with polydimethylsiloxane (PDMS) layer with eight wells was the structure of the device. Protein adding was done subsequent to the addition of suspension of the modified membrane and the signal was measured by a potentiometer. The detection limit was estimated to be 1fg/ml which was great (Mavrikou et al., 2020).

CONCLUSION

Recent developments in the field of biosensing technology, and molecular systems including LAMP microfluidics are promising to improve the quality and efficiency of diagnostics and will replace RT-PCR. Additional researches are needed to improve sensitivity, reproducibility, reliability of new detection methods. Furthermore, developed systems should analyze samples from diverse routes to confirm the results. The production of POC devices is still an urgent need to sensing of pathogens on-site without the need for trained personnel.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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SARS-CoV-2–nin xəstə üzərində COVID-19 testi ilə sürətli və sadə aşkarlanması

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COVID-19 bir ictimai sağlamlıq problemi olaraq dünya miqyasında yayılmışdır və tomoqrafiya görüntüsü, əks transkripsiya-polimeraza zəncir reaksiyası (RT-PCR), fermentə bağlı immunosorbent analizi (ELISA) və hüceyrə kultivasiyası daxil olmaqla müxtəlif metodların birləşməsi SARS-CoV-2-nin aşkar edilməsi və müəyyən olunması üçün inkişaf etdirilmişdir. COVID-19-un müalicəsi üçün spesifik antiviral maddələrin və ya peyvəndlərin olmaması səbəbindən onun erkən aşkarlanması və identifikasiyası çox vacibdir.Təcili yardım əməliyyatına başlamazdan əvvəl bioloji mayeləri nümunə olaraq istifadə edən səhiyyə işçiləri tərəfindən mütəmadi olaraq istifadə edilə bilən alternativ, həssas, sürətli xəstə üzərində aşkarlama vasitəsi arzuolunandır. Döngə vasitəçiliyi ilə izotermik gücləndirmə (LAMP) testləri, antikor testləri və mikrofluik RT-PZR cihazları (çip laboratoriyası) ilə daha təsirli diaqnostika və nəzarət texnologiyalarının inkişafı üzrə işlər davam etdirilir.POC diaqnostika, SARS-Cov-2 aşkarlanmasında ümidvericidir və alimləri konsepsiya

xaricində texnologiyaları inkişaf etdirməyə təşviq edir. SARS-CoV-2 üçün inkişaf etdirilmiş əks transkripsiya LAMP (RT-LAMP) metodu, bir saatdan az müddət ərzində tüpürcək nümunələrində belə virusu aşkar edə bilər. "Lab-on-a-chip" qurğusu kiçik ölçülü mikrosxem, mikrokanal, mikroelektrodlar və mikroqızdırıcıdan ibarətdir. Hüceyrə lizisi, DNT ekstraksiyası və PZR amplifikasiyası mərhələləri bu mikroçiplərə inteqrasiya edilə bilər.

Açar sözlər: *COVID-19, RT-PZR, polimer çip, termosikl, fövqəladə hallar, biosensorlar*

Быстрое и простое обнаружение SARS-CoV-2 с тестированием COVID-19 в присутствии пациента

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COVID-19 как проблема мирового общественного здравоохранения распространился по всему миру. Для обнаружения и идентификации SARS-CoV-2 разработаны различные методы, включая томографию, полимеразную цепную реакцию с обратной транскрипцией (RT-PCR), иммуноферментный анализ (ELISA) и культивирование клеток. Из-за отсутствия специфических противовирусных агентов или вакцин для лечения COVID-19 раннее обнаружение и идентификация имеют жизненно важное значение. Желательно было бы, чтобы медицинские работники, использующие в качестве образца биологические жидкости, перед началом оказания скорой медицинской помощи (POC) имели в наличии альтернативный чувствительный инструмент быстрого обнаружения, который мог бы регулярно ими использоваться. В настоящее время предпринимаются усилия по разработке более эффективных технологий диагностики и наблюдения с использованием тестов с петлевой изотермической амплификацией (LAMP), тестирования на антитела и применением микрофлюидных устройств RT-PCR (Lab-on-a-chip). POC-диагностика перспективна в обнаружении SARS-Cov-2 и побуждает ученых, совершенствующих технологии, выходить за пределы концепции. Метод обратной транскрипции LAMP (RT-LAMP), разработанный для SARS-CoV-2, может обнаружить вирус даже в образцах слюны менее чем за час. Устройства типа «лаборатория на чипе» содержат микросхему небольшого размера, микроканал, микроэлектроды и микронагреватель. В эти микрочипы можно интегрировать этапы лизиса клеток, экстракции ДНК и ПЦР-амплификации. Благодаря быстрому обнаружению, небольшому объему образца и интеграции с ПЦР в портативной крошечной системе, эти устройства являются многообещающими для обнаружения SARS-CoV-2. Необходимо повысить достоверность и чувствительность всех вышеупомянутых методов для использования образцов слюны; в случае улучшения они могут предоставить возможность обнаружения вируса в слюне без периода ожидания и сложной аналитической инфраструктуры.

Ключевые слова: *COVID-19, ОТ-ПЦР, полимерный чип, термоциклер, аварийные ситуации, биосенсоры*

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Clinical characteristics, risk factors and outcome of the mild and moderate COVID-19 infection

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The aim of this study was to present our personal experience on the basis of the results of the treatment of patients with COVID-19 in our clinic. Clinical results of COVID-19 patients treated and observed by authors at the AMU Surgical Hospital were investigated. Patients' demographics, the severity of infection, co-morbidities, clinical signs, viral examination, lung X-ray and CT, complications, treatments and their results were analyzed. Diagnosis and treatment of COVID-19 were carried out under the recommendations of TABIB and WHO. Antiviral Arbidol (Umifenovir) and Vitamin C were mainly used for the treatment of patients of the mild group (stable vital functions, normal saturation, no pneumonia). Arbidol, vitamin C, inhalation, prone position and antibiotics were used in the middle group (with symptoms of pneumonia, and not need oxygen therapy). Treatment was carried out for 11-14 days. Clinically improved patients with positive dynamics on X-ray and negative results on repeated PCR examinations were discharged from the hospital and sent for the supervision of an outpatient doctor. A total of 77 patients were under our supervision, of which 58 had mild and 19 had moderate COVID-19 infection. Of these patients, 57 were women and 20 were men, with an average age of 47.5 (18-84). Patients over 50 years of age accounted for 45.4%, and over 60 years of age for 15.5%. The average age was 45.6 % among mild patients and 53.8% among the moderate patients. The proportion of men in the moderate group increased in comparison with the mild group (from 19% to 47.4%). Concomitant diseases were found in 34 (44.2%) patients, asthma, pregnancy, epilepsy, viral hepatitis, cirrhosis, coronary heart disease, coronary stent, psychiatric illness, chronic kidney failure, bed sickness were observed besides smoking (11.7%), hypertension (9.1%) and diabetes (6.5%). In the moderate group, concomitant diseases were observed more in comparison with the mild group (39.7% and 57.9%). The most common clinical presentations were loss of smell and taste (67.5%) which were followed by cough (57.1%), fever (42.8%), shortness of breath and difficulty swallowing (24.6%). Mortality was not observed, complications were observed in 5 patients (6.5%), and all of these patients had concomitant diseases. Analysis of patients with mild and moderate COVID-19 infection allows us to come to the following primary conclusions: weakness, loss of smell and taste, and cough are the most common presenting symptoms; age over 60 years, age, diabetes, hypertension, smoking and chronic liver disease are aggravating risk factors; inhalation and prone position seem to be useful in moderate patients.

Keywords: *Covid 19, symptoms, demographics, concomitant diseases, treatment, prone position*

INTRODUCTION

The COVID-19 infection outbreak in China in December 2019 and rapidly spreading worldwide caused more than 60 million infections and 1,200 000 deaths of people. During this period, more than 40,000 infections and 1200 deaths were registered in our country. It should be noted that about 250 of 3000 clinical trials are registered regarding COVID-19 in the world have been completed. Unfortunately, these studies sometimes produce conflicting results (Pundi et al., 2020). Therefore, there

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is a need to continue research, including the study of regional features of the disease.

The aim of the study was to present our personal experience regarding the results of mild and moderate COVID-19 patients treated in our hospital.

MATERIALS AND METHODS

Results of the patients with diagnosis of COVID-19 who followed by authors during April-May 2020 at the Azerbaijan Medical University Surgery Hospital were analyzed. Demographics (age, sex), severity of infection, comorbidity, symptoms, viral examination, lung X-ray and CT, complications, treatments and outcomes were analyzed.

The diagnosis of COVID-19 was confirmed according to WHO recommendations, and the patients have been hospitalized if they had positive PCR test for COVID-19.

The treatment approach was selected according to severity and was based on WHO and TABIB `s protocols (Table 1). The management of mild patients was consist of in-hospital follow-up, antiviral, vitamin and symptomatic treatments. Moderate patients were also monitored in the ward, and in addition to antiviral, vitamin, and symptomatic treatments the inhalations, prone position and antibiotics (azithromycin, fluoroquinolones, cefalosopirns, ampicillin) were used to them. Severe and critical patients were treated in the intensive care unit.

Patients whose condition worsened during treatment were transferred to the intensive care unit.

The treatment was carried out within 12-14 days. Clinically improved patients with positive dynamics on X-ray and negative results on repeated PCR examinations were discharged from hospital and sent for supervision of an outpatient doctor.

Table 1. Severity of COVID-19 infection and treatments.

Severity	**Explanation**	**Treatment**
Mild	No or weak signs of systemic inflammation No clinical signs of pneumonia	Arbidol (Umifenovir) 200 mg 3 times a day Infusion -Ringer 500 ml Vitamin C 50 mg Symptomatic
Moderate	Symptoms of systemic inflammation. Clinical and imaging sings pneumonia No need for continouse oxygen therapy Saturation at rest and in room air ≥93%	Arbidol (Umifenovir) 200 mg, 3 times Infusion -Ringer 500 ml Vitamin C 50 mg Symptomatic Antibiotics Inhalation Prone position
Severe	Signs of systemic inflammation Signs of pneumonia Saturation is provided by non-invasive oxygen therapy (nasal warm oxygen flow or mask)	Arbidol Antibiotics Non-invasive oxygen therapy (nasal oxygen, oxygen mask) Inhalation Prone position
Critical	Organ failure Need for invasive oxygen therapy (mechanical ventilation, ECMO)	Supportive treatments Invasive Oxygen Therapy (Mechanical Ventilation, ECMO) Antibiotics Steroids Anticoagulant

Table 2. Characteristics of the patients.

Index	**Total**		**Mild**		**Moderate**	
	n	**%**	**n**	**%**	**n**	**%**
Total number of patients	77	100.0	58	75.3	19	24.6
Average age	47.5 (18-84)		45.6		53.8	
18-39	20	26.0	16	27.6	4	21.1
40-49	22	28.6	19	32.8	3	15.8
50-59	23	29.9	17	29.3	6	31.6
60-64	8	10.4	5	8.6	3	15.8
65 and over	4	5.2	1	1.7	3	15.8
Male	20	26.0	11	19.0	9	47.4
Female	57	74.0	47	81.0	10	52.6
Cases						
Medical institutions	41	53.2	35	60.3	6	31.6
Home	20	26.0	13	22.4	7	36.8
Workplace	7	9.1	5	8.6	2	10.5
Public place	5	6.5	2	3.4	3	15.8
Coming from abroad	4	5.2	4	6.9	0	0.0
Concomitant diseases	34	44.2	23	39.7	11	57.9
No	43	55.8	35	60.3	8	42.1
Asthma	1	1.3	0	0.0	1	5.3
Diabetes	5	6.5	5	8.6	0	0.0
Epilepsy	1	1.3	1	1.7	0	0.0
Pregnancy	3	3.9	2	3.4	1	5.3
HBV+HCV	1	1.3	0	0.0	1	5.3
Hypertension	7	9.1	3	5.2	4	21.1
Pneumonia	1	1.3	0	0.0	1	5.3
Smoking	9	11.7	3	5.2	6	31.6
Coronary stend	1	1.3	1	1.7	0	0.0
Ischemic heart disease	1	1.3	0	0.0	1	5.3
Chronic renal failure	1	1.3	1	1.7	0	0.0
Cirrhosis	1	1.3	0	0.0	1	5.3
Psychiatric	1	1.3	1	1.7	0	0
Symptoms						
Weakness, loss of smell and taste	32	41.6	32	55.2	0	0.0
Cough	5	6.5	5	8.6	0	0.0
Cough + loss of smell and taste	20	26.0	20	34.5	0	0.0
Cough and shortness of breath	19	24.7	0	0.0	19	100.0
No complaints	1	1.3	1	1.7	0	0.0
Normal fever	44	57.1	41	70.7	3	15.8
High fever	33	42.9	19	32.8	14	73.7
Signs of pneumonia on X-ray or CT	76	98.7		0.0	19	100.0
Complications	5	6.5	3	5.2	2	10.5
Liver failure	1	1.3	0	0.0	1	5.3
Respiratory failure	3	3.9	2	3.4	1	5.3
Premature delivery	1	1.3	1	1.7	0	0.0
Standard treatment	56	72.7	56	96.6	0	0.0
Standard + inhalation	19	24.7	0	0.0	19	100.0
Prone position	18	23.3	0	0	18	94.7
Supportive treatment	2	2.6	0	0.0	2	10.5

RESULTS

The results of total of 77 patients with mild to moderate severity are given in Table 2. There were 57 women and 20 men, an average age was 47.5. The oldest age was -84, the youngest age -18. Patients over 50 years old were 45.4%, and over 60 years old were 15.5%. The average age among the mild severity patients was -45.6, and moderate ones was 53.8 years.

From a sex point of view, women predominate in the general (74% women) and mild (81%) groups, but the proportion of men increases in the moderate group in comparison with the mild group (from 19% to 47.4%).

Patients get infection at medical institution, home, workplace, public place and foreign country. Most of patients infected in medical institutions (53.2%) and more than half of those were mild severity (60.3%).

Comorbidity were found in 34 patients (44.2%), including hypertension, diabetes, smoking, asthma, pregnancy, epilepsy, viral hepatitis, cirrhosis, coronary heart disease, benign psychiatric illness and chronic kidney failure. Smoking (11.7%), hypertension (9.1%) and diabetes (6.5%) were most common concomitant diseases. In comparison with mild group total comorbidity (39.7% vs 57.9%), smoking (5.2% vs 31.2%), and hypertension (5.2% vs 21.1%) were more common in moderate group. Three patients had pregnancy, two of them were mild and one of was moderate severity. Two of these pregnant women recovered, and premature delivery was in another. Both patients with chronic liver disease had moderate pneumonia, their condition worsened during treatment and were transferred to the intensive care unit.

Weakness and decreased or altered sense of smell and taste (67.5%) was most common presenting symptoms, followed by cough (57.1%), fever (42.8%), shortness of breath and difficulty swallowing (24.6%). Transient fever up to 38 C were noted in mild severity, but it was more than 38 C and lasted for several days in moderate severity patients.

Mortality was not observed, complications were observed in 5 patients (6.5%), and all of these patients had concomitant diseases. Respiratory failure was seen in 3 patients, one of them had been asthma, the second one had been hypertension and diabetes, and the third had been HBV + HCV and drug abuse. A patient in the early weeks of pregnancy had been premature delivery. In one patients with cirrhosis an acute decompensation was developed, which was treated with appropriate management.

DISCUSSION

Analysis of a series of patients with mild and moderate COVID-19 infection allows us to draw the following preliminary conclusions: weakness, loss of smell and taste and cough are the most common symptoms; age of 60 and more, concomitant diseases, diabetes, hypertension, smoking and chronic liver disease are risk factors that aggravate the disease; inhalation and prone position seem to be beneficial for moderate patients.

Other studies also have reported loss of taste and smell, cough, fever, and shortness of breath as a most common presenting symptoms in COVID-19 infection (Wiersinga et al.. 2020; Xie et al., 2020).

Although most studies have shown age, diabetes and hypertension, lung disease, and smoking as risk factors for aggravating COVID-19 disease, there are no consensus on the role of chronic liver disease (Wiersinga et al., 2020; Xie et al., 2020; Boettler et al., 2020) and extensive researches are needed. Antivirals, especially arbiodol and antibiotic therapy, have been reported no benefit in mild to moderate patients (Wiersinga et al., 2020). Although few studies have shown that inhalation and prone position beneficial (Guérin et al., 2013).

We should be noted that there are several limitations of this study. The first is the relatively small number of patients. Second one, there is no comparison group that is why it is difficult to determine effectiveness the treatments.

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Yüngül və orta dərəcəli COVID-19 infeksiyasının klinik xüsusiyyətləri, risk faktorları və nəticələri

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Tədqiqatın məqsədi klinikamızda müalicə alan COVİD-19 xəstələrinin müalicə nəticələri ilə əlaqədar şəxsi təcrübəmizi təqdim etməkdir. ATU klinikasında müəliflərin nəzarəti altına olan xəstələrin əldə olan məlumatları araşdırıldı. Xəstələrin demoqrafik göstəriciləri, yoluxduğu şərait, yanaşı xəstəlikləri, klinik əlamətləri, viral müayinə, ağciyər rentgeni və kompyuter tomoqrafiyası ağırlaşmalar, aldığı müalicələr və nəticələri təhlil edildi. COVİD-19 diaqnostikası və müalicəsi Ümumdünya Səhiyyə Təşkilatı və Tibbi Ərazi Bölmələrini İdarəetmə Birliyinin tövsiyələrinə əsasən aparılmışdır. Yüngül qrupda (həyatı funksiyaları stabil, saturasıyası normal, pnevmoniyası olmayan) əsas müalicə kimi antiviral Arbidol (Umifenovir) və Vitamin C verilmişdir. Orta qrupda (pnevmoniya əlamətləri olan, orqan yetməzliyi və oksigenoterapiyaya ehtiyac olmayan) Arbidol, vitamin C və simptomatik müalicələrə əlavə olaraq inhalyasiya, üzüaşağı vəziyyət və antibiotiklər verilmişdir. Müalicə 11-14 gün ərzində aparılmış, klinik olaraq düzələn, rentgendə müsbət dinamika və təkrari PCR müayinələrində neqativ nəticə gələnlər ambulator həkim nəzarətində evə yazılmışdır. Nəzarətimiz altında toplam 77 xəstə olmuş, bunlardan 58-i yüngül, 19-u isə orta dərəcəli COVİD-19 xəstələri olmuşdur. Xəstələrin 57-si qadın, 20-si kişi olmuş, ortalma yaş 47.5 (18-84) təşkil etmişdir. 50 yaşdan yuxarı xəstələr 45.4%, 60 yaşdan yuxarı isə 15.5 % təşkil etmişdir. Yüngül dərəcəlilər arasında ortalama yaş-45,6, orta dərəcəlilər arasında isə 53.8 olmuşdur. Yüngül qrupla müqayisədə orta qrupda kişilərin nisbəti artmışır (19%-dən 47.4%). Yanaşı xəstəliklərlər 34 (44.2%) xəstədə rastlanmış, siqaret (11.7%), hipertoniya (9.1%) və diabetlə (6.5%) yanaşı astma, hamiləlik, epilepsiya, viral hepatit, sirroz, koranar xəstəlik, stend, psixatrik xəstəlik, xroniki böyrək yetməzliyi, yataq xəstəliyi müşahidə edilmişdir. Yüngül qrupla müqayisədə orta qrupda yanaşı xəstəliklər (39.7% və 57.9%) daha çox rast gəlinmişdir. Klinik əlmatlər arasında ən çox halsızlıq və qoxu-dad hissiyyatının dəyişməsi (67.5%) rast gəlinmişdir, bunu öksürək (57.1%), yüksək hərarət (42.8%), nəfəs darlığı və udqunma çətinliyi (24.6%) izləmişdir. Letallıq rastlanmamışdır, ağırlaşma 5 xəstədə (6.5%) müşahidə edilmişdir və bu xəstələrin hamısında yanaşı xəstəliklər olmuşdur. Yüngül və orta dərəcəli COVİD-19 infeksiyası olan xəstə seriyasının təhlili aşağıdakı ilkin nəticələrə gəlməyə imkan verir: halsızlıq, qoxu-dad dəyişikliyi və öksürək ən çox rast gələn simptomlardır; 60-dan yuxarı yaş, yanaşı xəstəliklər, diabet, hipertoniya, siqaret və xroniki qaraciyər xəstəlikləri ağırlaşdırıcı risk amilləridir, orta dərəcəli xəstələrdə inhalyasiya və üzü aşağı yatızdırma müalicələri faydalı görünür

Açar sözlər: *Covid 19, simptomlar, demoqrafik göstəricilər, yanaşı gedən xəstəliklər, müalicə*

Клинические характеристики, факторы риска и исходы легкой и умеренной инфекции COVID-19

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В этом исследовании мы хотели представить наш личный опыт, приобретенный на основе результатов лечения пациентов COVID-19 в нашей клинике. В клинике АМУ были изучены данные, полученные от пациентов, находившихся под наблюдением авторов. Были проанализированы демография пациентов, условия инфицирования, сопутствующие заболевания, клинические признаки, вирусные обследования, рентгеноскопия легких и компьютерная томография, осложнения, методы лечения и их результаты. Диагностика и лечение COVID-19 проводились в соответствии с рекомендациями TABIB и ВОЗ. Антивирусные Arbidol (Umifenovir) и витамин С были применены в качестве основного лечения пациентов легкой группы (стабильные жизненные функции, нормальное насыщение, без симптомов пневмонии). Арбидол, витамин С, ингаляция, положение «лицом вниз» и антибиотики прописывались пациентам средней группы (с симптомами пневмонии, кислородная терапия не требовалась). Лечение проводилось в течение 11-14 дней. При исчезновении симптомов, положительной радиологической динамике, отрицательных результатах многократных ПЦР-обследований, больные были выписаны домой, где находились под наблюдением амбулаторного врача. 77 пациентов находились под нашим наблюдением, из них 58 больных были легкой, а 19 - средней степени тяжести. Среди пациентов 57 женщин и 20 мужчин, средний возраст которых составлял 47,5 лет (18-84 года). На долю пациентов в возрасте старше 50 лет приходилось 45,4%, а в возрасте старше 60 лет - 15,5%. Средний возраст составлял 45,6 для пациентов легкой и 53,8 для пациентов средней степени тяжести. Доля мужчин в средней группе увеличилась по сравнению с легкой группой (19% до 47,4%). Сопутствующие заболевания были обнаружены у 34 пациентов (44,2%), помимо курения (11,7%), гипертонии (9,1%) и диабета (6,5%), наблюдались астма, беременность, эпилепсия, вирусный гепатит, цирроз, сердечно-сосудистые заболевания, стенокардия, психические заболевания, хроническая почечная недостаточность, пролежни. В средней группе сопутствующие заболевания наблюдались в большей степени, чем в легкой группе (39,7% и 57,9%). Среди клинических признаков наиболее распространенными были усталость и потеря запаха и вкуса (67,5%), за ними следовали кашель (57,1%), лихорадка (42,8%), одышка и трудности с глотанием (24,6%). Смертность не наблюдалась, осложнения наблюдались у 5 пациентов (6,5%) и все эти пациенты имели сопутствующие заболевания. Анализ 9 пациентов с COVID-19 легкой и средней тяжести позволяет сделать следующие основные выводы: наиболее распространенными симптомами являются слабость, потеря запаха и вкуса, кашель; возраст (более 60 лет), диабет, гипертония, курение и хронические заболевания печени - усугубляющие факторы; ингаляция и положение «лицом вниз» полезны для больных средней тяжести.

Ключевые слова: *Covid 19, симптомы, демографические показатели, сопутствующие заболевания, лечение*

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Prevalence and fatality rates of COVID-19 in the elder age groups of Azerbaijan population

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The research aimed to study the age-related pattern of infection, and death rate among the confirmed positive cases of coronavirus (COVID-19) infection in people aged 60 and over. The statistical data on the prevalence and fatality rate of COVID-19 were provided by the Gerontological Center (Baku, Azerbaijan) and the Polyclinic of the Central Hospital in Lankaran, located in the south-eastern part of Azerbaijan. The analysis of statistical material has shown that the prevalence rate for COVID-19 is the highest for the age group of 60-75 years. It was also found that the fatality rate among the people aged 60+ with coronavirus infection was age-associated, and was the highest in the 80-89 age group. The obtained findings are consistent with the statistical data for COVID-19 prevalence and fatality rates for other countries and are indicative of a higher risk of death from coronavirus infection in older people.

Keywords: *COVID-19, coronavirus infection, age groups, Azerbaijan population*

INTRODUCTION

Since the coronavirus outbreak to this day, the COVID-19 pandemic has claimed the lives of more than one million people and infected more than 40 million people in 190 countries (https://worldometers.info). The number of the infected is increasing, and there are warning signs of new waves. According to the World Health Organization, the largest number of people infected with coronavirus is reported in the USA (8,489,786), India (7,606,256), Brazil (5,264,293), Russia (1,147,954) and Spain (1,015,795). At the same time, the fatality rate for these countries has the following values: USA - 2.7%, India - 1.5%, Brazil - 2.9%, Russia - 1.7%, Spain - 3.34%.

Officially, the spread of COVID-19 in Azerbaijan began on February 28, 2020, when the first case (a Russian citizen who crossed the border between Azerbaijan and Iran) in the country was registered by the newly established Operational Headquarters under the Cabinet of Ministers of the Republic of Azerbaijan (https://koronavirusinfo.az). According to official statistics, 45,879 COVID-19 cases, 635 deaths and 40,272 recoveries were registered in Azerbaijan as of October 20, 2020 (https://www.worldometers.info). The fatality rate of the infection in the country is 1.4% and this figure is lower in comparison with data for other countries, including those with more developed economies (https://worldometers.info).

Since the early days of the pandemic, COVID-19 has had the greatest impact on older people worldwide in terms of number of cases, hospitalizations, and deaths (Sudharsanan et al., 2020; Liu et al., 2020). Several months after the beginning of the coronavirus pandemic, older people have been continuing to be at highest risk for severe illness and death from COVID-19 (Omori et al., 2020). Age is undoubtedly the most important factor in predicting the probability of survival of patients

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with COVID-19 (Davies et al., 2020). The pandemic is far from over, and therefore, there is a need for more effective ways to minimize the death rates. In this regard, Azerbaijan has promptly responded to the pandemic by timely imposing lockdown, and the number of new cases and deaths is relatively low at present as compared to other countries, however, the scale of the problem and factors of vulnerability cannot be underestimated. Therefore, the main objective of the present study was to identify the most vulnerable population groups for further adherence to COVID-19 prevention measures in a comparative perspective.

MATERIALS AND METHODS

The statistical data on prevalence and fatality rate of COVID-19 among people aged 60 and over were provided by the Gerontological Center (Baku, Azerbaijan) and the Polyclinic of the Central Hospital (Lankaran, Azerbaijan). In our study, we used the data which cover the period 07.06.2020-11.08.2020. The statistical analysis was performed with application of Microsoft Excel and a software package Statistica for Windows.

RESULTS AND DISCUSSION

According to the official statistics by the Gerontological Center, the population of Baku is 2,293,047, of which 319,788 (13.9%) are people aged 60+, with age group of 60-75 making up 11.4% (261,046) of Baku total population at age of 60+. The 75-90 age category represents 2.4% (55,640) of the population of Baku, while the people aged 90+ make up only 0.14% (3,102). Over the mentioned period, 2,469 people aged 60+ tested positive for COVID-19. As can be seen from the above data, this figure is 0.11% of the total population of Baku and 0.77% of the number of people aged 60+. Among the surveyed people with confirmed diagnosis of COVID-19, 54.8% were women (1,354) and 45.2% were men (1115). In our work, it was shown that 1,801 people out of those, who tested positive for COVID-19, recovered, while the fatality rate was 3.7%. Important to note that this figure is 2.4 times higher than that characterizing the general fatality rate of the coronavirus infection in Azerbaijan (https://worldometers.info). At the time of our study, 602 people were kept under medical supervision and were receiving treatment. With consideration age classification by the World Health Organization, our work shows that the COVID-19 cases most often occurred in the age group of 60-75. Their number is 2209, which is 89.5% of the total number of those with a positive test outcome. These people make up 0.85% of the total population of Baku in this age category. Of them, 1010 are men (45.7%) and 1199 are women (54.3%). In the 76-90 age group, 249 people tested positive for COVID-19, which in turn amounts to 10.1% of all people with a positive test outcome for COVID-19 and 0.45% of the population of Baku in this age category. Of them, 89 (35.7%) are men and 160 (64.3%) are women. As to age category of long-livers (age 90+), in total 11 long-livers were recorded as infected by coronavirus in Baku, which make up 0.4% of the total number of subjects with a positive test result and 0.35% of Baku residents of this age. Of them, 4 are men (36.4%) and 7 (63.6%) are women.

In general, our work shows that among the representatives of the population of Baku, who aged 60+, the COVID-19 cases most often occurred in the age group of 60-75 years (Fig. 1). It should also be noted that that age group contains the largest number of people in comparison with the other groups studied in our work. In addition, in each studied age group, the number of women who tested positive for COVID-19 is higher than the corresponding number of men.

The quantitative indices of COVID-19 cases among people aged 60+ in various districts of Baku during the survey period are presented in Table 1. As can be seen from the table, the proportion of infected people aged 60+ to the total population of the corresponding district was 0.1±0.01% (σ: 0.04), which indicates a relatively low number of cases of infection with COVID-19 in each district of Baku. The proportion of COVID-19 cases among people of the above-mentioned age to the population of the same age of the corresponding district was equal to 0.73 ± 0.08% (σ: 0.27), which, in our opinion, may be associated with an increase in the risk of contracting COVID-19 with age. Obviously, the Pirallahi district shows the smallest number of COVID-19 cases among people aged 60+ in comparison with the other districts of Baku. This fact is presumably due to geographical location of the district in one of the Absheron peninsula islands which prevents the spread of infection.

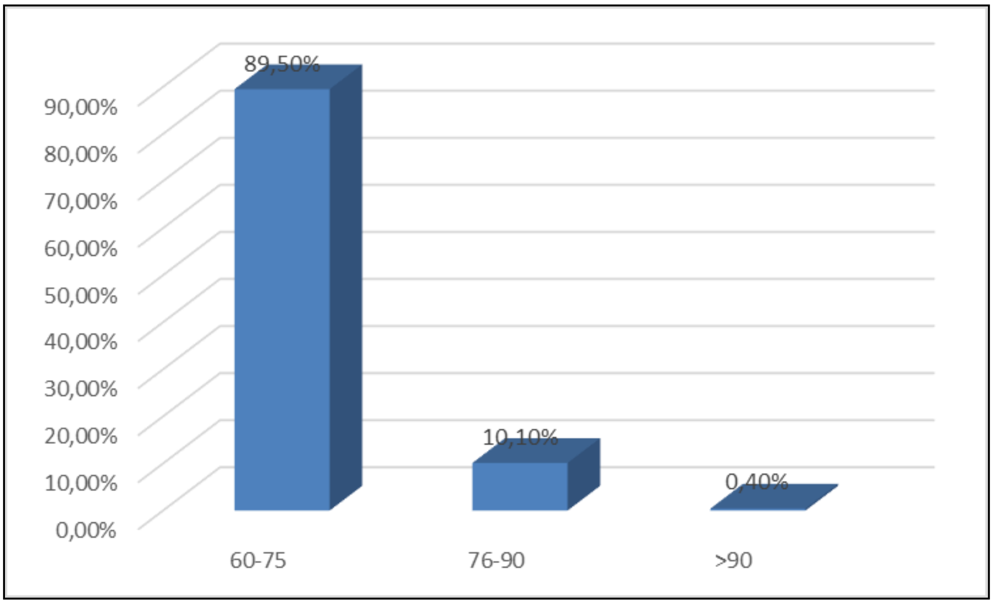


Fig. 1. COVID-19 cases in Baku during 07.06.2020-11.08.2020
(data provided by Baku Gerontological Center)

Table 1. COVID-19 situation by the districts of Baku (07.06.2020-11.08.2020)

Districts of Baku	COVID-19 cases among people aged 60+	Proportion of COVID-19 cases among people aged 60+ to all cases	Proportion of COVID-19 cases among people aged 60+ to the total population of the district	Proportion of COVID-19 cases among people aged 60+ to the population aged 60+ of the district
Binagadi	409	16.6%	0.15%	1.2%
Yasamal	314	12.7%	0.13%	0.79%
Khatai	307	12.4%	0.11%	0.74%
Surakhani	271	11%	0.12%	0.9%
Narimanov	259	10.5%	0.14%	1.0%
Sabunchi	224	9.1%	0.08%	0.7%
Nasimi	212	8.6%	0.1%	0.64%
Nizami	121	4.9%	0.06%	0.4%
Garadakh	119	4.8%	0.09%	0.87%
Sabail	119	4.8%	0.12%	0.75%
Khazar	108	4.4%	0.06%	0.51%
Pirallahi	6	0.2%	0.03%	0.21%

Table 2. COVID-19 situation in Lankaran by age

Age	Male	Female	Deaths	Deaths (male)	Deaths (female)
60-69	38	55	5	3	2
70-79	8	5	2	2	-
80-89	4	4	2	2	-
≥90	-	1	-	-	-

The statistical data on the situation with COVID-19 prevalence and death rate among the elder population (age groups of 60+) in Lankaran (a city located 276 km away from Baku) were provided by the local Polyclinic of the Lankaran Central Hospital. The analysis of statistical material has showed that among the people aged 60+ (60-69, 70-79, 80-89, ≥90), COVID-19 cases most often occurred in the 60-69 age category (Table 2),

while the fatality rate was 7.8%. This figure is 2.1 times higher than the analogous index obtained from the data provided by the Gerontological Center in Baku and 5.6 times higher than the total death rate of the coronavirus infection in Azerbaijan.

Along with the above, it was also found that the fatality rate among the people aged 60+ with coronavirus infection was age-associated, and it was highest in the 80-89 age group (Fig. 2).

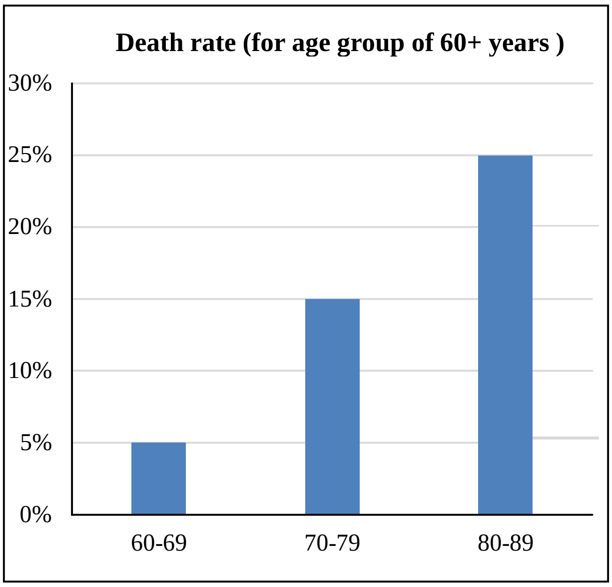


Fig. 2. The death rate among people with COVID-19 in Lankaran by age groups

Our findings serve as one more evidence of the wide-spread opinion by the experts that the elderly population is at the greatest risk for severe illness from COVID-19. At the same time, the case of the full recovery from COVID-19 for female patient at age of 96, which was recorded in Lankaran, is indicative of role of other factors conditioning the final outcome of the disease.

In conclusion, with due consideration of the huge negative impact of the outbreak of coronavirus there is an urgent need to identify the age groups at the highest risk in terms of the disease outcome. As show numerous studies age is one of the major factors conditioning the COVID-19- associated death rate (Zhou et al., 2020). This is because aging is associated with certain changes in lung function during the period of pulmonary infection, and thus age-related differences in responsiveness and tolerance become apparent and lead to worse clinical outcomes in older people (Liu et al., 2020). Immune aging is one of the first signs of the aging process. So, both innate and adaptive parts of the immune system are impaired with age. In addition, the elderly have a constant production of inflammatory mediators and cytokines (Perrotta et al., 2020). It is known that people under 65 have a very low risk of dying from COVID-19 even in the epicenters of a pandemic, and deaths of people under 65 without underlying predisposing conditions are extremely rare (Ioannidis et al., 2020). Thus, comparing the findings obtained in our study with the data on the other countries, special attention should be paid to the strategies aimed at protecting older people as the most vulnerable population group.

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Azərbaycan populyasiyasında yuxarı yaş qruplarının COVID-19 xəstəliyinə yoluxma və ölüm halları

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Tədqiqatın əsas məqsədi COVID-19 koronavirus infeksiyasına müsbət testlərin statistik məlumatları əsasında Azərbaycan populyasiyasının yuxarı yaş kontingentinin (60+) müxtəlif qruplarında yoluxmaların və ölüm hallarının rastgəlmə səviyyələrinin təhlili olmuşdur. Bundan ötrü Bakı şəhər herontoloji mərkəzindən və Azərbaycanın cənub-şərq bölgəsində yerləşən Lənkəran şəhərinin mərkəzi xəstəxanasının poliklinikasından təqdim edilən statistik məlumatlar istifadə olunmuşdur. Statistik materialın təhlili göstərir ki, 60 və yuxarı yaş kateqoriyasına daxil olan şəxslər üçün ən çox yoluxma halları 60-75 yaş qrupuna təsadüf edir. Eləcə də müəyyən edilmişdir ki, koronavirus infeksiyasına müsbət test nəticələri təsdiq olunmuş 60+ yaşlı şəxslərdə ölüm halları göstəricisi yaşla bağlılıq nümayiş etdirir və ən yüksək göstərici 80-89 yaş qrupunda aşkar edilib. Əldə nəticələr yuxarı yaş qruplarında koronavirus infeksiyasına yoluxanlar arasında yüksək ölüm riskinin olmasına dəlalət edən digər ölkələrin statistik məlumatlarına uyğundur.

Açar sözlər: *COVID-19, koronavirus infeksiyası, yaş qrupları, Azərbaycan populyasiyası*

Заболеваемость и смертность от COVID-19 в старших возрастных группах Азербайджанской популяции

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Основной задачей исследования было выявление распространенности заражения и уровня смертности на основе статистических данных о положительных тестах на коронавирусную инфекцию COVID-19 у лиц в возрасте 60 лет и старше. Для этой цели были использованы статистические данные о заболеваемости и смертности от COVID-19, представленные Бакинским геронтологическим центром и поликлиникой Центральной больницы г.Ленкорань (юго-восточный регион Азербайджана). Анализ статистического материала показал, что у лиц в возрастной категории 60 лет и старше наибольшая встречаемость случаев заражения COVID-19 имела место в возрастной группе 60-75 лет. Также было установлено, что у людей 60 лет и старше с положительным результатом на наличие коронавирусной инфекции, показатель смертности имеет возраст-зависимый характер, а наибольший показатель был обнаружен в возрастной группе 80-89 лет. Полученные результаты подтверждаются данными по другим странам, что свидетельствует о высокой степени риска летального исхода у лиц старшего возраста с коронавирусной инфекцией.

Ключевые слова: *COVID-19, коронавирусная инфекция, возрастные группы, Азербайджанская популяция*

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Clinical features and characteristics of myocardial injure in COVID-19 (data from multicenter studies)

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In patients with COVID-19 caused by coronavirus-2 (SARS-CoV-2) and proceeding with the severe acute respiratory syndrome, studies were carried out to study myocardial damage, determine its nature and clinical significance. In an international, multicenter study, cardiovascular pathologists assessed cardiac tissue after necropsies in 21 COVID-19 patients. The presence of myocarditis was determined by the identification of multiple foci of inflammation with associated damage to myocytes, and the composition of the inflammatory cells was analyzed using immunohistochemistry. Other forms of acute damage and inflammation of myocytes, as well as damage to the coronary arteries, endocardium and pericardium, have also been described. Lymphocytic myocarditis occurred in 3 (14%) cases. Increased infiltration of interstitial macrophages was observed in 18 (86%) cases. In four cases, there was mild pericarditis. Acute damage to right ventricular myocytes, most likely due to stress/overload, occurred in four cases. In COVID-19, myocardial damage has been reported, including elevated serum troponin levels and acute heart failure with decreased ejection fraction. There was a slight trend towards higher serum troponin levels in patients with myocarditis compared with patients without myocarditis. With SARS-CoV-2, interstitial macrophages increase in most cases, and in a small proportion of cases, multifocal lymphocytic myocarditis. Other forms of myocardial injury may also occur. The risk of hospital death among patients with severe COVID-19 can be predicted from markers of myocardial damage and has been significantly associated with older age, inflammatory response, and concomitant cardiovascular disease.

Keywords: *Coronavirus Disease 2019 (COVID-19), SARS-CoV-2, myocardial injury, myocarditis, lactate dehydrogenase, cardiac troponin I, creatine kinase (-MB), myoglobin*

INTRODUCTION

Coronavirus disease 2019 (COVID-19) caused by Coronavirus-2 (SARS-CoV-2) and a severe acute respiratory syndrome is primarily a respiratory disease (Du et al., 2020; Fauci et al., 2020; Fauci et al., 2020), but systemic and cardiovascular damage can occur. SARS-CoV-2 can cause an intense release of cytokines and chemokines, which can lead not only to vascular inflammation and instability of atherosclerotic plaques, but also to myocardial inflammation. Acute heart injury with elevated serum troponin levels is the most frequently reported cardiac abnormality in COVID-19, reported in about 8-12% of patients, and elevated troponin levels are associated with increased mortality in COVID-19 patients (Madjid et al., 2020; Shi et al., 2020). In addition, a small proportion of patients develop acute heart failure with reduced ejection fraction, which raises clinical concerns about myocarditis (Ramirez et al., 2008; Wang et al., 2020; Zhou et al., 2020). Possible mechanisms for increasing troponin levels in these patients include ischemia, stress cardiomyopathy, microvascular thrombosis and secondary effects of systemic inflammation.

Direct viral myocardial infection is another possible route of myocardial injury. The unique affinity of SARS-CoV-2 for the host angiotensin-

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converting enzyme receptor 2 increases the likelihood of direct viral infection of the vascular and myocardial endothelium, so that in some patients, myocardial damage associated with COVID-19 may represent viral myocarditis (Zhou et al., 2020). Any of these mechanisms can also exacerbate underlying cardiovascular diseases (Zhu et al., 2020).

There have been several reports describing cardiac histology in a small number of COVID-19 patients, mostly associated with endomyocardial biopsy and limited autopsy sampling (Gallagher, Ferrario and Tallant, 2008). A study was also conducted aimed at assessing heart damage in patients with COVID-19 and determining the frequency and type of myocarditis, as well as other forms of acute heart damage. Several studies have reported clinical and laboratory results related to cardiovascular injury in patients with COVID-19 infection (Corrales-Medina et al., 2013; Huang et al., 2020; Ji et al., 2020; Oudit et al., 2009).

Accumulating evidence indicates that myocardial injury is a complication associated with COVID-19, with a prevalence of 7.2% to 12%. Notably, the American College of Cardiology Clinical Bulletin highlighted the cardiac implications of COVID-19. This suggests that patients with cardiovascular disease are at higher risk and recommends triage and treatment as a priority (Wang et al., 2020). The researchers determined the predictive value of myocardial scores in relation to hospital death and studied the characteristics and potential causes of myocardial damage in cases of severe COVID-19.

This review summarized the laboratory findings and mechanism of cardiac dysfunction associated with COVID-19 infection.

MATERIALS AND METHODS

A working group on COVID-19, led by the Society for Cardiovascular Disease and the European Association for Cardiovascular Disease, was requested to provide information regarding cardiac pathology obtained from successive autopsies performed at their facilities on COVID-19 patients. Inclusion criteria were: positive nasopharyngeal swab for SARS-CoV-2, clinical diagnosis of COVID-19, and autopsy with cardiac examination by a cardiologist from the working group, including collection of myocardial samples (left ventricle, septum, and right ventricle) and epicardial coronary arteries. All sequential autopsies meeting these criteria were included. An international multicenter study assessed cardiac tissue after autopsy in 21 COVID-19 patients. Information provided included the presence of myocarditis and other types of inflammation and trauma identified on hematoxylin and eosin (H&E) -stained heart sections. The degree of stenosis of the coronary arteries and the presence of destroyed plaques were also presented. Evaluation of the myocardium by electron microscopy and evaluation of immunohistochemical spots on CD68, CD3, CD4 and CD8 were provided. For immunohistochemical staining, the number of cells with the greatest inflammation, stained in a field with a high magnification × 400 was counted. All immunohistochemical staining was performed on conventional automatic diagnostic devices for immunohistochemical staining. The presence of left ventricular fibrosis was assessed on a semi-quantitative scale: mild (<10% of the myocardial area); moderate (10-25% of the area of the myocardium); Serum troponin levels were obtained at selected institutions using the following tests: high sensitivity troponin T, normal <15 ng/L (n=6), high sensitivity troponin I, normal <35 ng/L (n=6), high sensitivity troponin I , norm <19.8 ng / l (n=4). Electrocardiographic changes were considered new if they were not present on previous electrocardiograms during the current or previous hospitalization. All electrocardiographic changes were obtained on a 12-lead ECG.

For the purposes of this study, myocarditis was defined as the presence of an inflammatory infiltrate associated with damage to myocytes not caused by any other cause that was present in multiple foci. The views expressed by them do not necessarily reflect the views of all members of the Society for Cardiovascular Disease or the European Association for Cardiovascular Disease.

Another retrospective study included laboratory-confirmed COVID-19 patients admitted to Wuhan University Renmin Hospital located in Wuhan, Hubei Province. This study was approved by the National Health Commission of China and the Institutional Review Board of Renmin Hospital, Wuhan University (Wuhan, China). Written informed consent was rejected by the Hospital Ethics

Commission appointed to treat emerging infectious diseases.

The severe COVID-19 patients included in this study were diagnosed according to the COVID-19 Diagnostic and Treatment Guidelines (Study Sixth Edition) published by the National Health Commission of China on February 18, 2020. Cases in this study included severe illness characterized by any of the following: respiratory rate> 30 / min; oxygen saturation ≤93%; PaO 2 / FiO 2 ratio ≤ 300 mmHg.; respiratory failure requiring mechanical ventilation; shock; or respiratory failure in combination with other organ failure requiring intensive care. Cases younger than 18 years of age and missing cardiac biomarkers, including cardiac troponin I (cTnI) levels, were excluded.

The patients were grouped according to whether they died (death group) or survived (survivor group). The study resulted in hospital mortality rates and clinical outcomes were determined based on information stored in the hospital's real-time medical record system.

Myocardial injury was defined as an increase in the level of cardiac biomarkers cTnI in the blood above the 99th percentile of the upper reference limit (Huang et al., 2020) ARDS was defined in accordance with the Berlin definition (Wan et al., 2020). The date of onset of the disease was considered the day when the symptom was noticed.

Studied creatinine kinase-myocardial band (CK-MB), myoglobin (MYO) and cTnI. The normal CK-MB reference range is 0–5 ng/mL; the normal MYO reference range is 0-110 µg/L; the normal cTnI reference range is 0–0.04 ng/ml and the minimum detectable concentration (analytical sensitivity) is 0.006 ng / ml. Normal range (NT-proBNP is 0-900 pg/ml.

A retrospective design was used in 20 studies to investigate cardiac damage associated with severe outcome and death in patients with COVID-19 infection in China (Jing et al., 2020; Gaze, 2020). Two studies used a prospective design (Ruan et al., 2020; Wang et al., 2020) Study sample size ranged from 10 to 645 patients (mean age of severe patients: 60.95 years, mean age of patients without severe degree: 46.95 years).

Another study was conducted in New York from February 27, 2020 to April 12, 2020, with an enrollment of 2,736 patients. in patients within 24 hours after hospitalization was measured troponin-I (normal value <0.03 ng/ml) (Anuradha et.al., 2020).

Statistical analysis

Descriptive statistics were obtained for all study variables. Continuous data are expressed as median (interquartile range, IQR). Categorical data is expressed in proportions. All categorical variables were compared for study results using Fisher's exact test or x-square test, and continuous variables were compared using Student's t-test or Mann-Whitney U test. Logistic regression analysis was performed to determine predictors of myocardial injury. Cases of missing biomarker data were excluded using statistical software. Data were analyzed using SPSS 25.0 (IBM, Chicago, Illinois). Statistical charts were created using Prism 5 (GraphPad), Minitab (version 18) and Python. For all statistical analyzes, P<0.05 was considered significant. statistical analysis.

RESULTS

21 cases met the study criteria. These autopsies were performed in March or April 2020 at Asienda Ospedaliera University of Padua, Sant'Orsola-Malpighi University Hospital in Bologna, Massachusetts General Hospital in Boston, University of Amsterdam and the Mayo Clinic in Rochester, Minnesota. For all 21 patients, COVID-19 was the leading cause of death. Causes of death: acute respiratory distress syndrome (ARDS, n=15), viral pneumonia (n=4), cardiogenic shock (n=1), and cardiac arrest (n=1). Eighteen patients died in intensive care. Two of the patients received veno-venous extracorporeal membrane oxygenation.

Since the last hospitalization, serum high-sensitivity troponin T or I levels have been available in 16 patients with a mean peak value of 56 ng/L in the range of 2.8–2494 ng/L. Troponin levels were altered in 11 of these 16 patients. Five out of 16 patients received renal replacement therapy.

Electrocardiographic changes were detected in 12 patients, including new-onset atrial fibrillation (n=5), partial right bundle branch block (n=2), nonspecific T-segment changes (n=3), transient ST-segment elevation (n=1), premature ventricular

contractions (n=1) and ST segment depression (n=1). The duration of atrial fibrillation was 12 hours in one patient, 2 days in three patients, and 3 days in one patient. Limited bedside transthoracic echocardiography was performed in five patients without myocarditis. 16 patients received one or more COVID-19 drugs, including hydroxychloroquine / chloroquine (n=15), azithromycin (n=8), atorvastatin (n=4), inhaled nitric oxide (n=2), lopinavir / ritonavir (n=6), oseltamivir (n=2), remdesivir (n=1), tocilizumab (n=3), and sarilumab (n=1).

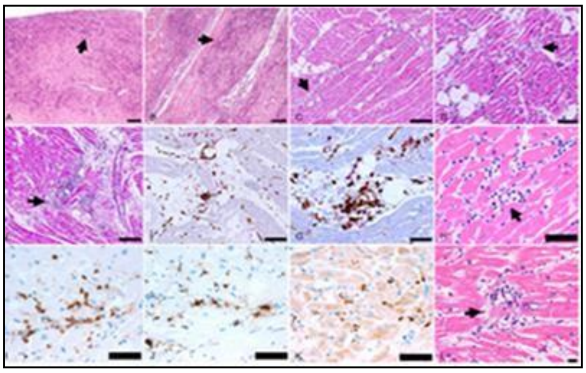


Fig. 1. Spectrum of myocarditis in COVID-19 patients. (A and B) Biventricular multifocal/diffuse lymphocytic myocarditis (arrows) with extensive myocyte injury in an 86-year-old man with previously undiagnosed cardiac amyloidosis (H&E×50). (C–G) Biventricular multifocal lymphocytic myocarditis (arrows) with myocyte injury in a 64-year-old man, who developed atrial fibrillation 2 days before death (C, H&E ×100; D, H&E ×200; E, H&E ×100; F, double immunostaining CD68 brown/CD3 red, ×200; G, double immunostaining CD4 brown/CD8 red, ×400). (H–K) Biventricular multifocal lymphocytic myocarditis (arrow) in a 59-year-old man (H, H&E ×400; I, CD3 immunostaining brown, ×400; J, CD68 immunostaining brown, ×400; K, CD4 immunostaining brown, ×400). (L) Focal myocardial lymphocytic infiltration with myocyte injury (arrow) in a 70-year-old man (H&E ×400). Scale bars represent 500 μm (A, B), 200 μm (C, E), 100 μm (D, F), 50 μm (G–K), and 20 μm (L).

At autopsy, no thromboembolism of the main pulmonary arteries was found. An average of 20 complete myocardial blocks (range 5–29 blocks) were histologically examined and immunohistochemical staining of inflammatory cell markers was assessed in all cases. Myocarditis was detected in three cases (Fig. 1). Myocarditis was multifocal in all three cases with left and right ventricular involvement, but in one case, right ventricular predominance was present. In all three cases, myocarditis was classified as lymphocytic, containing a significant number of CD3 + T lymphocytes and a significant proportion of CD68 + macrophages, without eosinophils, giant cells, or granulomas. In two cases, lymphocytes were CD4 +, and in one case, lymphocytes were with a predominance of CD8 +. In addition to the three cases of multifocal myocarditis, there were six cases of focal enlargement of interstitial T lymphocytes in the myocardium, with or without focal damage to myocytes, with the number of focal T cells ranged from 22 to 65 per field × 400 at high magnification.

In 18 (86%) cases, there was a relatively widespread increased infiltration of interstitial macrophages into the myocardium without clearly associated damage to myocytes, affecting both the left and right ventricles (Fig. 2). These diffuse macrophage infiltrates were observed in two out of three patients with myocarditis and in 16 out of 18 patients without myocarditis. In a third patient with myocarditis, the inflammatory infiltrates were relatively extensive, making it difficult to identify a distinctive single macrophage infiltrate. The average density of macrophages in these cases was 44 cells in a high power field (range 20–177). Mild pericarditis from the epicardium (visceral pericardium) was present in four cases.

Acute damage to myocytes in the right ventricle, most likely due to stress / overload, occurred in four cases and was characterized by acute coagulation necrosis of myocytes, mainly in the subendocardial region, with staining of necrotic myocytes for the complement component C4d.

When comparing cases of myocarditis and patients without myocarditis, the densities of CD3 + lymphocytes and CD68 + macrophages were higher in patients with myocarditis than in patients without myocarditis, but there were no differences in the density of inflammatory cells between the left and right ventricles. There were no differences between the two groups in duration of symptoms, length of hospital stay, age, history of hypertension, history of diabetes, history of previous immunosuppression, history of smoking, or history of previous cardiovascular disease (Table 1). There was also no discernible difference between the two groups in COVID-19 related treatment.

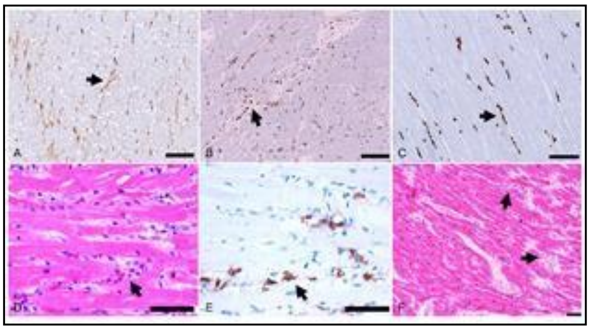


Fig. 2. Increased interstitial macrophages. The majority of patients showed increased interstitial macrophages without associated myocyte injury (arrows). (A) A 50-year-old man, (CD68 immunostaining, ×100). (B) A 44-year-old man, (double CD68 brown/CD3 red immunostaining, ×100). (C) A 64-year-old man (CD68 immunostaining, ×200). (D and E) A 60-year-old man (D, H&E, ×400; (E) CD68 immunostaining, ×400). (F) A 73-year-old woman with increased cells within the myocardial interstitium (H&E, ×100). Scale bars represent 200 μm (A, B), 100 μm (C, F), and 50 μm (D, E).

For 16 patients with documented troponin levels, there was no significant difference in serum troponin levels between patients with and without myocarditis (Table 1). While the patient with the highest troponin level (2494 ng/L) did have myocarditis, the patient with the second highest troponin level (702 ng/mL) did not have myocarditis, but had acute right ventricular myocardial injury, most likely as a result of stress. All three patients with myocarditis had troponin levels ≥60 ng/L compared with only 38% of patients without myocarditis (P = 0.20). In addition, all three patients with myocarditis had serum troponin levels ≥60 ng/mL with new ECG changes, compared with only 2 (15%) of 13 patients without myocarditis (P = 0.02).

A total of 2253 confirmed COVID-19 cases were initially screened from January 1 to February 23, 2020 at Wuhan University's Renmin Clinical Hospital. 671 cases (death, 62; survivors, 609) with severe COVID-19 were included in the study. These patients had a mean age of 63 years (IQR, 50–72 years), 48% of patients were male, and the mean time from symptom onset and admission to follow-up was 23 days and 17 days, respectively.

During hospitalization, 95.5% of patients received oxygen therapy; however, the use of extracorporeal membrane oxygenation and continuous renal replacement therapy was rare. The proportion of using antiviral treatment was 96.4% in the included patients, and 59.5%, 56.5% and 54.2% of patients, respectively, received intravenous immunoglobulin, glucocorticoids and antibiotics.

The deceased patients were older than the survivors and more often than males (all P <0.001, Table 1).

The main cause of death was a rapid deterioration in respiratory function, followed by cardiovascular complications. Table 2 summarizes the distribution of death-related complications in the included patients, including ARDS (98.4%), acute respiratory failure (90.3%), acute myocardial injury (30.6%), acute heart failure (19.4%), multiple organ failure syndrome (9.7%), shock (6.5%) and sudden death (1.6%).

Table 1. Characteristics of patients

Treatment, n (%)	All patients	Death	Survivors	p
Oxygen inhalation	527 (78,5)	16 (25,8)	511 (83,9)	<0,001
Non-invasive ventilation	76 (11,3)	17 (27,4)	59 (9,7)	<0,001
Invasive mechanical ventilation	36 (5,4)	29 (46,8)	7 (1,1)	<0,001
Extracorporeal membrane oxygenation	2 (0,3)	2 (3,2)	0 (0,0)	0,008
Continuous renal replacement therapy	4 (0,6)	4 (6,5)	0 (0,0)	<0,001
Antiviral agent	647 (96,4)	58 (93,5)	589 (96,7)	0,267
Immunoglobulin	399 (59,5)	55 (88,7)	344 (56,5)	<0,001
Glucocorticoids	379 (56,5)	53 (85,5)	326 (53,5)	<0,001
Antibiotic	364 (54,2)	49 (79,0)	315 (51,7)	<0,001

Table 2. Cause of death of included patients.

Complications	n (%)
Acute Respiratory Distress Syndrome	61 (98,4)
Acute respiratory failure	56 (90,3)
Acute myocardial injury	20 (30,6)
Acute heart failure	12 (19,4)
Multiple organ failure syndrome	6 (9,7)
Shock	4 (6,5)
Sudden death	1 (1,6)

106 patients (15.8%) had myocardial injury in all enrolled patients on admission. Patients with myocardial injury had an older age, more comorbidities and more laboratory abnormalities than patients without myocardial injury. Patients who died were more likely to suffer from myocardial injury during hospitalization compared with survivors (75.8% versus 9.7%; P <0.001). The contour plot of the characteristics of the distribution of myocardial parameters showed that these biomarkers were higher among the deceased patients. From admission to death, cardiac scores showed dynamic change in the death group, especially CK-MB and cTnI levels. Since most patients were not followed up during hospitalization, the serial biomarker results were based on a very small subgroup.

Cumulatively evaluated 17 studies 6, 8-16, 18, 20-22, 24-26 of 2,467 patients infected with COVID-19 (severe patients = 1095 and non-severe patients = 1372), higher serum lactate dehydrogenase levels were shown to be (weighted average difference = 108.86 U / L, 95% confidence interval (CI) = 75.93 to 141.79, p <0.001, I 2 = 85.4%, p heterogeneity <0.001) and creatine kinase-MB (weighted average difference = 2.60 U / L, 95% CI = 1.32–3.88, p <0.001, I 2 = 0.0%, p heterogeneity = 0.517) associated with a significant increase in the severity of COVID-19 infection. The pooled results showed that serum creatine kinase levels (weighted mean difference = 15.10 U / L, 95% CI =0.93 to 31.12, p=0.065, I2= 46.9%, p heterogeneity =0.058), cardiac troponin I (weighted average difference = 4.05 pg / ml, 95% CI= -0.20 to 8.30, p=0.062, I 2=0.0%, p-heterogeneity = 0.591) and myoglobin (weighted average difference =21.40 ng / ml, 95% CI= -0.22 to 43.02, p=0.052, I2=29.3%, p heterogeneity = 0.243) did not have a significant relationship with the severity of the disease.

Six studies 7, 14, 17, 19, 23, 27, including a total of 1217 patients with COVID-19 infection (survivor=365 and survivors=852), reported mortality as an outcome measure. The pooled results showed that higher serum lactate dehydrogenase levels (weighted mean difference = 213.44 U/L, 95% CI =129.97-296.92, p <0.001, I 2 = 90.4%, p heterogeneity < 0.001), Creatine kinase (weighted average difference = 48.10 U/L, 95% CI=0.27–95.94, p=0.049, I2=85.0%, p heterogeneity=0.001), cardiac troponin I (weighted average difference=26.35 pg / ml, 95% CI=14.54 to 38.15, p<0.001, I2=4.1%, p heterogeneity = 0.352) and myoglobin (weighted average difference =159.77 ng / ml, 95% CI= 99.54 to 220.01, p<0.001, I 2 =0.0%, p heterogeneity=0.409) were associated with a significant increase in mortality from COVID-19 infection.

In a study conducted in New York City, 506 (18.5%) patients died during hospitalization. A total of 985 (36%) patients had an increased troponin concentration. After adjusting for disease severity and related clinical factors, even minor myocardial injuries (eg troponin I> 0.03–0.09 ng/ml; n= 455; 16.6%) were significantly associated with death (adjusted hazard ratio: 1, 75; 95%). CI: 1.37 to 2.24; p <0.001), while higher amounts (e.g. troponin I> 0.09 ng / dl; n = 530; 19.4%) were significantly associated with higher risk (adjusted HR: 3.03; 95% CI: 2.42 to 3.80; p <0.001) (Anuradha et al., 2020).

DISCUSSION

Detailed information on cardiac autopsy in patients who have died from COVID-19 is currently very limited. Despite the high mortality rate worldwide, only a few studies with a small number of patients still provide information on cardiac disease in these patients. (Li et al., 2003; Mo et al. 2020; Xu et al., 2020). Some of these studies used limited diagnostic approaches such as biopsy. This training has several limitations. Molecular analysis for the virus in the myocardium was not performed. this study is still relatively small and not complete enough to identify and exclude differences between groups. The definition of a COVID-19 diagnosis in SARS-CoV-2 infected patients may have varied across settings in this multicenter study. A

small number of patients underwent only limited bedside echocardiography. The electrocardiographic data were based on documented clinical observations and were not obtained in a standardized manner. This study was retrospective and not all cases were collected for histology in the same way. For this study, the authors used a rigorous criterion for multiple lesions associated with myocardial injury to diagnose myocarditis. Thus, there is great confidence that three patients meeting this criterion have myocarditis. There were six additional cases with lymphocytic infiltrates but no or only focal myocyte injury. In some previous studies not related to COVID-19, in particular with the participation of endomyocardial biopsy, this pathology was considered as myocarditis. and the results cannot be easily generalized to all patients dying from COVID-19.

One of the key pathological discoveries in this series of studies is that patients dying from COVID-19 often have infiltration of interstitial myocardial macrophages with an average of 44 cells per field at × 400 magnification, without damaging myocytes, affecting 86% of patients. In fewer cases, true multifocal lymphocytic myocarditis affects 14% of patients. Compared to the previous SARS-CoV virus, the inflammatory heart changes seen with COVID-19 appear to be more severe overall. An early autopsy of patients who died from SARS showed that 35% of patients could detect SARS-CoV in myocardial tissue by PCR, and this subgroup of patients had a degree of myocardial macrophage infiltration comparable to that of 86% of patients. cases of COVID-19 in this series (Zhang et al., 2020). The median age of patients in the previous SARS study was 68 with 45% of men, compared to a median age of 69 with 71% of men for COVID-19 patients in this study. The mechanisms underlying this macrophage infiltration are currently unclear, but the study by Oudit et al. it has been suggested that SARS-CoV-induced myocardial inflammation is mediated predominantly by macrophages. Also, this study did not show that SARS-CoV is associated with an increase in lymphocytic infiltrates or multifocal myocarditis, as with SARS-CoV-2 infection.

Although there was a slight upward trend in troponin levels in patients with myocarditis in these studies, myocarditis does not fully explain the increased troponin levels seen in patients with COVID-19. Other forms of myocardial injury, such as right ventricular stress, clearly contribute to the increased troponin levels in these patients. All patients with multifocal myocarditis in this series had new changes in electrocardiography, including atrial fibrillation in two cases and new ST segment depression associated with chronic atrial fibrillation in a third case. Given that most patients with COVID-19 have an increase in the number of macrophages in the heart, it can also be difficult to determine with imaging studies which of these patients actually has lymphocytic myocarditis. Although electron microscopy has reported the presence of the virus in cardiac macrophages (Lo et al., 2020.). However, so far, electron microscopy in this series of patients has been performed only in three cases without real myocarditis.

Preliminary observations in the literature, together with those in this series, suggest that myocardial injury, with or without cardiac depression, in these patients may be due to an etiology other than viral myocarditis. Acute damage to myocardial tissue may be associated with increased cytokines, hypoxemia, right ventricular tension, and thrombotic complications. In some patients in this series, both myocardial microvascular thrombi and right ventricular deformity were observed. Thus, the term myocarditis should be used to describe patients with elevated troponin levels in the presence of COVID-19 using more specific diagnostic tests such as endomyocardial biopsy and / or cardiac magnetic resonance imaging.

Cardiac abnormalities associated with SARS-CoV-2 infection were found to be more severe than those associated with a previous SARS-CoV outbreak. Under the conditions of SARS-CoV-2 infection, in most cases, the number of interstitial myocardial macrophages increases, and in a small part of cases, multifocal lymphocytic myocarditis. These patients also present with other forms of myocardial injury, such as right ventricular deformity.

The main findings of this study are as follows: myocardial injury is not uncommon among patients with severe COVID-19, especially among those who die; elevated levels of myocardial markers predict the risk of death in hospital; and advanced age, inflammatory response, and concomitant cardiovascular disease are associated with myocardial injury in COVID-19 patients (Chen et al., 2020; Corrales-Medina et al., 2012).

Increases in cTnI and CK-MB can predict the risk of death. Notably, these single cut-off values may include a range of individuals with normal levels of myocardial markers at admission. While it is premature to say whether these patients are doomed to poor outcomes, as only about 30% of people have died from myocardial damage, this plays an early warning role for COVID-19 deaths when these rates exceed thresholds.

Circulating inflammatory mediators (i.e. cytokines and/or endotoxins) or direct viral invasion of cardiomyocytes, or both, can lead to myocardial damage in COVID-19. The novel coronavirus was recently reported to use angiotensin converting enzyme II (ACE2) as a cell entry receptor, and SARS-CoV was found in the heart of 35% of subjects, suggesting that SARS-CoV is capable of infecting the myocardium through ACE2 receptors (Li et al., 2003). While it can be argued that direct invasion of SARS-CoV-2 into cardiomyocytes underlies heart failure, a recent pathological study documented scant interstitial mononuclear inflammatory infiltrates in cardiac tissue without significant myocardial damage in a COVID-19 patient (Mo et al., 2020). With limited evidence, whether SARS-CoV-2 can directly damage the heart remains to be proven.

Previous data have demonstrated that risk factors for cardiac complications associated with pneumonia include older age, preexisting cardiovascular disease, and a greater severity of pneumonia at presentation (Guan et al., 2019). However, about one third of transient cardiac complications occur in patients without a history of heart disease. The SARS study also found that reversible left ventricular damage is common, even among those without underlying heart disease (Thygesen et al., 2018). However, sufficient evidence is needed to clarify whether the effects of COVID-19 and SARS on the myocardium differ. In the present study, the deceased patients had a higher proportion of both myocardial injury and concomitant cardiovascular diseases, suggesting that cardiac complications with underlying cardiovascular disease or risk usually coexist and develop to an irreversible outcome. Alternatively, the systemic inflammatory response to pneumonia may also increase the inflammatory activity in coronary atherosclerotic plaques, making them unstable and prone to rupture. Consequently, the presence of preexisting cardiovascular disease or associated risk factors can exacerbate myocardial damage, which therefore cannot be ignored when treating COVID-19. In general, patients with COVID-19 and myocardial injury are discouraged from actively participating in emergency intervention strategies due to the existing risk of cross-infection, and for most moderate to moderate myocardial injuries, the standard integrated management process is usually based on risk stratification and patient classification (Tikellis and Thomas, 2012).

Studies have confirmed the hypothesis that heart damage is associated with severe outcome and death in patients with COVID-19 infection. It should be noted that this study is the first meta-analysis to assess the relationship between serum cardiac biomarker levels and the severity of COVID-19 infection.

It is known that advanced age (≥65 years), male sex and the presence of comorbidities such as hypertension, diabetes, chronic obstructive pulmonary disease and cancer are the main risk factors for death from COVID-19 (Wang et al., 2020). The presence of myocarditis and heart damage (defined by elevated cardiac troponin I levels above the upper limit of the 99th percentile) are other independent risk factors associated with mortality (Ranieri et al., 2012).

COVID-19 can exacerbate underlying cardiovascular diseases and / or cause new heart conditions. Previous studies have shown that the incidence of acute heart damage in severe COVID-19 patients and deaths ranges from 5% to 31% and 59% to 77%, respectively (Anuradha et al., 2020; Fauci, Lane and Redfield, 2020; Han et al., 2020). The auxiliary mechanisms include hemodynamic changes, induction of procoagulant factors, and systemic inflammatory reactions, which are mediators of atherosclerosis, directly contributing to plaque rupture through local inflammation, which predisposes to thrombosis and ischemia (Davidson and Warren-Gash, 2019; Han et al., 2020; Hoffmann et al., 2020).

In addition, ACE2, the COVID-19 receptor, is expressed on vascular endothelial cells and myocytes (Smeeth et al., 2004), so there is at least a theoretical potential for direct virus damage to the cardiovascular system. In theory, this could have a potential impact on patients taking angiotensin-converting enzyme inhibitors, resulting in a greater

risk of contracting COVID-19 and an increase in the severity of the disease (Chen et al., 2020).

Other putative mechanisms of COVID-19-related heart damage include a cytokine storm mediated by an increase in the production of pro-inflammatory cytokines by innate immunity after infection with COVID-19, and hypoxia caused by excessive intracellular calcium leading to myocyte apoptosis.

Interstitial mononuclear inflammatory infiltrates in the myocardium have been reported in deaths from COVID-19 (Li et al., 2003). In addition, cases of myocarditis with reduced systolic function have been reported following infection with COVID-19 (28). Heart damage is likely associated with ischemia and / or infectious myocarditis and is an important prognostic factor in patients with COVID-19 infection. COVID-19 affects the myocardium and causes myocarditis (Li et al., 2003).

Studies of cardiac biomarkers indicate a high prevalence of cardiac injury in deaths from COVID-19 infection (Li et al., 2003; Ranieri et al., 2012). Mortality was significantly higher in patients with high serum levels of lactate dehydrogenase, cardiac troponin I, creatine kinase, and myoglobin. The mechanism by which cardiac biomarkers increase in COVID-19 infection is not fully understood. The underlying pathophysiology suggests a cardio-inflammatory response, as many patients with severe COVID-19 infection have a concomitant rise in cardiac biomarkers and acute phase reagents such as C-reactive protein (Chen et al., 2020). An increase in cardiac biomarkers with other inflammatory biomarkers increases the likelihood that this reflects a cytokine storm and may clinically present as fulminant myocarditis.

For patients admitted to hospital with COVID-19, in addition to routine clinical assessment, a standardized measurement of cTn to detect myocardial injury along with other inflammatory (e.g., C-reactive protein, ferritin, IL6, and procalcitonin) and thrombotic (D-dimeric) markers can make it easier to understand whether patients are in stage I (early infection), stage II (pulmonary phase), or stage III (hyperinflammatory phase). In addition, as an ongoing prognostic marker, initial cTn measurement can aid triage of patients, and subsequent serial measurements can help identify low-risk patients with stable concentrations or high-risk patients with increasing patterns. The latter may require additional assessments (Yader and Allan, 2020).

LIMITATIONS

The research carried out has some limitations. First, the interpretation of the results may be limited by the small sample size of a total of 2350000 COVID-19 patients worldwide, the current sample size is still small to avoid statistical bias as much as possible. Second, due to the presence of unmeasured or unknown factors that influence the outcome, the causes of death or myocardial damage can be underestimated by multivariate regression analysis. The study did not include data such as body weight, body mass index and smoking history, which are potential risk factors for disease severity.

CONCLUSIONS

Myocardial injury is not a rare complication among patients with severe COVID-19, especially among those who die. In a meta-analysis of patients with confirmed COVID-19, heart damage assessed by serum analysis (lactate dehydrogenase, cardiac troponin I, creatine kinase (-MB), and myoglobin) was associated with severe outcome and death from COVID-19 infection. CTnI and CK-MB levels predict the risk of hospital death, and myocardial injury is associated with older age, inflammatory response, and concomitant cardiovascular disease. With the rapid development of COVID-19 around the world and a deeper understanding of the mechanisms of heart damage in patients with COVID-19 infection, cardiac biomarkers can be used as an indicator of improved response due to cardioprotective intervention or as an indicator. deterioration of the clinical course.

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COVID-19 zamanı miokardın zədələnməsinin klinik xüsusiyyətləri (Çox mərkəzli tədqiqatların məlumatları)

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Koronavirus-2-nin (SARS-CoV-2) səbəb olduğu COVID-19 xəstəliyi özünü kəskin tənəffüs sindromunun inkişafı ilə göstərir. Bu xəstələrdə miokardın zədələnmə xüsusiyyətlərini öyrənmək və onun klinik əhəmiyyətini təyin etmək məqsədi ilə təqqiqat işləri aparılmışdır. Beynəlxalq çoxmərkəzli tədqiqatlarda ürək-damar patologiyalarının araşdırılması zamanı COVID-19 olan 21 xəstədə anatomik təşrifdən sonra ürək toxuması tədqiq edilmişdir. Kardiomiositlərin zədələnməsini göstərən çoxsaylı iltihab ocaqlarının aşkarlanmasına və immunohistoloji müayinə vasitəsi ilə iltihab hüceyrələrinin tərkibinin analizinə əsasən miokarditin olması müəyyənləşdirilmişdir. Eyni zamanda kardiomiositlərin kəskin zədələnməsinin və iltihabın digər növləri, həmçinin koronar arteriyaların, endokard və perikard zədələnmələri də tədqiq edilmişdir. Limfositar miokarditə 3 (14%) halda rast gəlinmişdir. İnterstisial makrofaqların artan infiltrasiyası 18 (86%) halda müşahidə edilmişdir. 4 xəstədə yüngül dərəcəli perikardit qeyd edilmişdir. Böyük ehtimalla stress/həddindən artıq yüklənmə səbəbindən 4 xəstədə sağ mədəcik miositlərinin kəskin zədələnməsinə rast gəlinmişdir. COVID-19-da serum troponin səviyyəsinin yüksəlməsi və atma fraksiyasının azalması ilə müşayət olunan kəskin ürək çatışmazlığı hallarına da rast gəlinmişdir. SARS-CoV-2 zamanı əksər hallarda interstisial makrofalar artır, az bir halda isə multifokal limfositar miokardit inkişaf edir. Ağır COVID-19-lu xəstələrdə xəstəxanadaxili ölüm riski miokardın zədələnmə markerlərinə əsasən proqnozlaşdırıla bilər və bu risk ahıl yaş, iltihabi reaksiya və yanaşı ürək-damar xəstəliyinin olmasından əhəmiyyətli dərəcədə asılıdır.

Açar sözlər: *Koronavirus xəstəliyi 2019 (COVID-19), SARS-CoV-2, miokardın zədələnməsi, miokardit, laktat dehidrogenaza, troponin I, kreatininfosfokinaza, mioglobin*

Клинические особенности и характеристика повреждения миокарда при COVID-19 (данные многоцентровых исследований)

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Известно, что COVID-19 – тяжелое острое респираторное заболевание, вызванное коронавирусом SARS-CoV-2. Пациенты с коронавирусной инфекцией исследовались с целью изучения повреждения миокарда, определения его характера и клинической значимости. В международном многоцентровом исследовании сердечно-сосудистых патологий состояние сердечной ткани было оценено после вскрытия 21 пациента с COVID-19. Исследования были направлены на выявление наличия миокардита, множественных очагов воспаления с ассоциированным повреждением миоцитов, состав воспалительных клеток анализировали с помощью иммуногистохимии. Также были описаны другие формы острого повреждения и воспаления миоцитов, поражение коронарных артерий, эндокарда и перикарда. Лимфоцитарный миокардит имел место в 3 (14%) случаях. Повышенная инфильтрация интерстициальных макрофагов наблюдалась в 18 (86%) случаях. В четырех случаях наблюдался перикардит легкой степени. Острое повреждение миоцитов правого желудочка, вероятнее всего из-за напряжения / перегрузки, имело место в четырех случаях. При COVID-19 также отмечалась острая сердечная недостаточность, сопровождаемая повышенным уровнем тропонина и сниженной фракцией выброса. В большинстве случаев при SARS-CoV-2 были увеличены интерстициальные макрофаги, а в небольшой части случаев - мультифокальный лимфоцитарный миокардит. Риск госпитальной смерти среди пациентов с тяжелой формой COVID-19 можно предсказать по маркерам повреждения миокарда, и это в значительной степени связано с пожилым возрастом, воспалительными реакциями и сопутствующими сердечно-сосудистыми заболеваниями.

Ключевые слова: *Коронавирусное заболевание 2019 г. (COVID-19), SARS-CoV-2, повреждение миокарда, миокардит, лактатдегидрогеназа, сердечный тропонин I, креатинкиназа (-MB) и миоглобин*

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Comparative studies of E, M and N structural proteins of SARS-CoV, SARS-CoV-2, pangolin CoV and bat CoV

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During the last two decades, humanity has been plagued by 3 coronavirus diseases, although the human coronaviruses were discovered over 50 years ago. The latest coronavirus disease discovered in 2019 (COVID-19) is caused by human Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). ***A question arises:*** **what could be the reason for such activation of coronaviruses in recent years? To answer this question, at least, it is necessary to clarify (1) the history and origin of these viruses, and (2) molecular mechanisms how they very easily and rapidly enter into host cells and cause multifaceted serious disorders. In this study, we compared the structural proteins E, M and N from SARS-CoV-2, SARS, bat and pangolin CoVs. The most striking fact firstly discovered in this study is that the relative proportion of the synonymous substitution rates in M and N proteins of the SARS-CoV-2 and pangolin CoV are significantly higher than the corresponding characteristics for other CoVs studied. This finding puts several intriguing questions on the emergence and the duration of divergence of the SARS-CoV-2.**

Keywords: *Coronavirus, COVID-19, E protein, M protein, N protein, pangolin, bat, origin of the SARS-CoV-2, synonymous mutations, non-synonymous mutations*

INTRODUCTION

Coronaviruses (CoVs), enveloped RNA viruses, cause diseases of wide range in mammals and birds (Fehr and Perlman, 2015; Rabi et al., 2020). In 2002-2003, the highly pathogenic Severe Acute Respiratory Syndrome coronavirus, SARS-CoV, was discovered in China. Later (2012), the Middle Eastern Respiratory Syndrome Coronavirus (MERS-CoV) emerged in Kingdom of Saudi Arabia. At last, in December 2019, a new and extremely dangerous disease, COVID-19, associated with the most known pathogenic SARS coronavirus, SARS-CoV-2, was fixed in China (Shi and Hu, 2020). To date (November 19, 2020), there have been about 56,000,000 confirmed cases of COVID-19, including over 1,344,000 deaths (https://www.who.int/emergencies/diseases/novel-coronavirus-2019). Thus, during last two decades, the humanity has been plagued by 3 coronavirus diseases, although the human coronaviruses were discovered over 50 years ago (McIntosh et al., 1967; McIntosh, 1974). Therefore, a natural question arises: what could be the reason for such activation of coronaviruses in recent years? To answer this question, it is necessary to determine the approximate date and origin of the virus in the human body. Moreover, if we understand the events that led to the emergence of human coronaviruses, we can also predict and prevent new pandemics.

Bats are currently considered as one of the potential natural reservoirs of various viruses, including SARS-CoV and MERS-CoV (for a review see: Cui et al., 2019). Indeed, studies indicate that many coronaviruses are capable of interspecies transmission (Tang et al., 2015). In partic-

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ular, some bat coronaviruses and SARS-CoV can use the same receptor to enter cells (Hu et al., 2015; Menachery et al., 2015). However, recent findings indicate that SARS-CoV-2-like CoVs might originate from pangolin species (Lopes et al., 2020; Malaiyan et al., 2020; Tang et al., 2020; Zhang et al., 2020).

Coronaviruses have a positive-sense RNA genome of ~30 kb, with a 5′-cap and a 3′ poly (A)-tail structure. This structure allows RNA to serve as a direct mRNA for synthesis of the viral polypeptides (Fehr and Perlman, 2015; Hu et al., 2015). In particular, the SARS-CoV-2 genome (29,880 bp) encodes four main structural, spike (S), membrane (M), envelope (E), and nucleocapsid (N) proteins, and nonstructural (3-chymotrypsin like protease, papain-like protease, and RNA-dependent RNA polymerase) proteins (for review see: Huang et al., 2020).

The SARS-CoV-2 entrance into the host cell is initiated by interactions between the ~150 kDa S protein and its receptor, the angiotensin-converting enzyme 2 (ACE2). This protein is composed of the N-terminal S1 (13-685 aa) and the C-terminal S2 (686-1270 aa) subunits gained by a cleavage of the primary S protein by a host cell furin-like protease. The host ACE2 is recognized and bound by the S1 subunit, while the S2 is required mainly for a fusion of viral and host cell membranes. Thus, protein S appears to be an important determinant of CoV pathogenesis and resistance to infection in the body.

The small (~8-12 kDa) and probably transmembrane E protein is found in small quantities within the virion. Although the E proteins have been found to vary greatly in different CoV groups, they share a common architecture. In contrast to other structural proteins, recombinant viruses lacking the E protein are not always lethal. The E protein is mostly involved in assembly and release of the virus, but is also required for pathogenesis (Fehr and Perlman, 2015; (Bianchi et al., 2020; for a review see: Satarker et al., 2020).

The N protein (~49.5 kDa) is an important antigen for CoV, which participates in RNA package and virus particle release (Zeng et al., 2020). N protein is second most abundant viral protein, and is expressed during the early stages of infection. It is composed of two separate domains, an N-terminal domain (NTD) and a C-terminal domain (CTD), both of which are capable of binding RNA *in vitro*. However, the optimal RNA binding function of the N protein is supposed to be required for both domains. This protein helps to enter the host cell and interact with cellular processes after the virus fusion. (Huang et al., 2004; V’kovski et al., 2019). The SARS-CoV-2 and SARS-Cov N protein sequence shows about 90% similarity (Gralinski and Menachery, 2020). N protein of SARS-CoV promotes the activation of cyclooxygenase-2 (COX-2) and causes inflammation in the lungs (Yan et al., 2006). It also participates in the inhibition of a phosphorylation of the B23 protein, which is involved in the development of the cell cycle (Zeng et al., 2008), as well as in inhibition of the viral proteins degradation (Wang et al., 2010). Moreover, N protein restricts immune responses in the body against the viral infections via inhibition of the type I interferon (Lu et al., 2011).

The M protein (∼25-30 kDa) is the most abundant structural protein in the virion (Alsaadi and Jones, 2019). It contains 3 transmembrane domains and is required for the shaping and budding processes of CoVs (Bianchi, et al., 2020). Most M proteins do not contain a signal sequence, although they are co-translationally inserted in the endoplasmic reticulum membrane (Fehr and Perlman, 2015). Structural analysis of the M protein indicates its existence in two, long and compact, forms. This protein inhibits the Nuclear Factor kappa-light-chain-enhancer of activated B cells through interactions with I Kappa B Kinase and reduces levels of Cyclooxygenase 2, thus enhancing the proliferation of the viral pathogen (Fang et al., 2007). At last, the protein is known to be involved in the activation of beta-interferons (Satarker et al., 2020).

However, we are too far from understanding molecular mechanisms determining their host range and pathogenesis rate, supposed harmful side effects in the host organisms. In this sense, a comparative exploration of SARS-CoV-2, SARS-CoV and other related coronavirus genomes from human, bat and other species seems to be one of most efficient ways in understanding genetic bases of the CoV problem. In particular, the whole-genome sequencing and analysis data on SARS-CoV-2 from different populations are recently emerging (Munnink et al., 2020; Meredith et al., 2020).

In this study, the E, M and N proteins from SARS-CoV-2, SARS-Cov, bat CoV and pangolin CoV are compared. Below, we present and discuss results of these studies.

MATERIALS AND METHODS

For analysis, both CDS and protein sequences of the E, M and N proteins from the human, pangolin and bat CoVs, including SARS-CoV-2 (GenBank accession MN997409.1), SARS-CoV E (NC_004718.3), the pangolin CoV (MT040335.1), 9 strains of the bat CoVs (strain 273/2005: GenBank accession: ABG47063.1; 279/2005: ABG47072.1; Italy/206679-3/2010: AZF86133.1; Italy/206645-41/2011: AZF86121.1; Italy/3398-19/2015: AZF86127.1; Rm1/2004: ABD75325.1; Rp3/2004: AAZ67055.1; HKU9: YP_001039974.1; Vs-CoV-1: BBJ36014.1) were used.

A comparison of CDS and protein sequences was done by BLAST tool (Altschul et al., 1997). A multiple alignment of CDS and protein sequences, as well as the construction of the phylogenetic trees was performed by the Clustal Omega tool (Sievers and Higgins, 2014; Sievers and Higgins, 2018).

To investigate the statistical characteristics of variations, such as identities, synonymous and nonsynonymous substitutions, as well as insertions/deletions (Indels), as a new tool, MUTAN-2 was developed by I. Shahmuradov (unpublished). An output of the pairwise alignment of protein sequences by the Clustal Omega (in the FASTA format) and corresponding query CDS sequences serve as a source (input) data for this tool.

RESULTS AND DISCUSSION

Initially, using the BLAST and Clustal Omega tools, we compared E, M and N protein sequences from the human SARS-CoV-2 and SARS-CoV, as well the pangolin CoV and 9 strains of the bat CoV (see: Materials and Methods). Results of these comparisons are illustrated in Table 1, 2 and 3, as well as in Fig. 1, 2 and 3. Proteins E and M, as well as human and pangolin CoV proteins show significant (88% or higher) similarity.

However, only 4 (out of 9) strains (273/2005, 279/2005: Rm1/2004 and Rp3/2004) of bat CoV were found to have significant (88% or higher) similarity to the corresponding human and pangolin CoV proteins. The same results were obtained for S proteins (a paper on comparative studies of the CoV S-proteins was recently submitted elsewhere).

Table 1. Percent identity matrix for the *E* proteins from the human SARS-CoV and SARS-CoV-2, pangolin CoV and 9 strains of the bat CoV

	1	2	3	4	5	6	7	8	9	10	11	12
1. Bat (Italy-1)		100.0	70.67	16.00	20.00	16.67	16.67	16.44	16.44	17.81	17.81	17.81
2. Bat (Italy-3)	100.0		70.67	16.00	20.00	16.67	16.67	16.44	16.44	17.81	17.81	17.81
3. Bat (Italy-2)	70.67	70.67		18.67	22.67	15.28	15.28	15.07	15.07	16.44	16.44	16.44
4. Bat (HKU9)	16.00	16.00	18.67		20.25	25.68	25.68	26.67	26.67	26.67	26.67	26.67
5. Bat (Vs)	20.00	20.00	22.67	20.25		40.00	40.00	40.79	40.79	40.79	40.79	40.79
6. CoV-2	16.67	16.67	15.28	25.68	40.00		**100.0**	**96.00**	**96.00**	**94.67**	**94.67**	**94.67**
7. Pangolin	16.67	16.67	15.28	25.68	40.00	**100.0**		**96.00**	**96.00**	**94.67**	**94.67**	**94.67**
8. SARS	16.44	16.44	15.07	26.67	40.79	**96.00**	**96.00**		**100.0**	**98.68**	**98.68**	**96.05**
9. Bat (Rp3)	16.44	16.44	15.07	26.67	40.79	**96.00**	**96.00**	**100.0**		**98.68**	**98.68**	**96.05**
10.Bat (279)	17.81	17.81	16.44	26.67	40.79	**94.67**	**94.67**	**98.68**	**98.68**		**100.0**	**96.05**
11.Bat (Rm1)	17.81	17.81	16.44	26.67	40.79	**94.67**	**94.67**	**98.68**	**98.68**	**100.0**		**96.05**
12.Bat (273)	17.81	17.81	16.44	26.67	40.79	**94.67**	**94.67**	**96.05**	**96.05**	**96.05**	**96.05**	

Hereinafter, the following abbreviations are used: "Italy-1" for the Italy/206679-3/2010, "Italy-2" for the Italy/206645-41/2011, "Italy-3" for the Italy/3398-19/2015, "Vs" for the Vs-CoV-1, "Rm1", "Rp3" for the Rp3/2004, "273" for the 273/2005 and "279" for the 279/2005. A group of CoVs with the significant similarity of *E*-proteins are highlighted in grey. Here, as well as in Table 2 and 3, the SARS-Cov-2 and pangolin CoV similarity is marked in pink, similarity between SARS CoV and 4 bat CoVs (strains Rp3, 279, Rm1 and 273) is highlighted in red.

Table 2. Percent identity matrix for the *M* proteins from the human SARS-CoV, pangolin CoV and 9 strains of the bat CoV

	1	2	3	4	5	6	7	8	9	10	11	12
1. Bat (Italy-1)		100.0	88.11	28.05	27.60	29.55	29.55	29.09	29.09	29.09	31.22	32.72
2. Bat (Italy-3)	100.0		88.11	28.05	27.60	29.55	29.55	29.09	29.09	29.09	31.22	32.72
3. Bat (Italy-2)	88.11	88.11		29.09	29.55	30.45	30.45	30.45	30.00	30.00	31.96	35.94
4. CoV-2	28.05	28.05	29.09		**98.20**	**90.50**	**90.50**	**89.59**	**89.14**	**89.59**	38.91	40.37
5. Pangolin	27.60	27.60	29.55	**98.20**		**90.95**	**90.95**	**90.50**	**89.59**	**90.05**	38.91	39.91
6. Bat (279)	29.55	29.55	30.45	**90.50**	**90.95**		**100.0**	**97.29**	**95.93**	**97.29**	40.45	40.83
7. Bat (Rm1)	29.55	29.55	30.45	**90.50**	**90.95**	**100.0**		**97.29**	**95.93**	**97.29**	40.45	40.83
8. SARS	29.09	29.09	30.45	**89.59**	**90.50**	**97.29**	**97.29**		**97.74**	**97.29**	40.91	41.28
9. Bat (273)	29.09	29.09	30.00	**89.14**	**89.59**	**95.93**	**95.93**	**97.74**		**98.64**	40.91	40.83
10. Bat (Rp3)	29.09	29.09	30.00	**89.59**	**90.05**	**97.29**	**97.29**	**97.29**	**98.64**		40.91	40.83
11. Bat (HKU9)	31.22	31.22	31.96	38.91	38.91	40.45	40.45	40.91	40.91	40.91		41.28
12. Bat (Vs)	32.72	32.72	35.94	40.37	39.91	40.83	40.83	41.28	40.83	40.83	41.28	

A group of CoVs with the significant similarity of *M*-proteins are highlighted in grey.

Table 3. Percent identity matrix for the *N* protein from the human SARS-CoV, pangolin CoV and 9 strains of the bat CoV

	1	2	3	4	5	6	7	8	9	10	11	12
1. Bat (Italy-1)		98.38	64.11	23.69	27.96	27.90	27.27	27.27	27.59	27.59	27.27	27.90
2. Bat (Italy-3)	98.38		64.11	23.97	27.66	27.59	26.65	26.65	27.27	27.27	26.96	27.59
3. Bat (Italy-2)	64.11	64.11		25.07	29.97	30.37	29.14	29.45	30.06	29.45	29.45	29.75
4. Bat (HKU9)	23.69	23.97	25.07		39.11	44.30	44.92	44.92	44.70	44.81	43.04	43.77
5. Bat (Vs)	27.96	27.66	29.97	39.11		47.85	48.48	48.48	48.23	48.35	48.73	48.98
6. Bat (273)	27.90	27.59	30.37	44.30	47.85		**95.71**	**96.19**	**96.44**	**96.67**	**88.78**	**88.97**
7. Bat (279)	27.27	26.65	29.14	44.92	48.48	**95.71**		**99.52**	**97.14**	**97.38**	**88.76**	**89.18**
8. Bat (Rm1)	27.27	26.65	29.45	44.92	48.48	**96.19**	**99.52**		**97.62**	**97.62**	**89.00**	**89.42**
9. SARS	27.59	27.27	30.06	44.70	48.23	**96.44**	**97.14**	**97.62**		**98.10**	**89.74**	**89.93**
10. Bat (Rp3)	27.59	27.27	29.45	44.81	48.35	**96.67**	**97.38**	**97.62**	**98.10**		**89.26**	**89.93**
11. CoV-2	27.27	26.96	29.45	43.04	48.73	**88.78**	**88.76**	**89.00**	**89.74**	**89.26**		**93.76**
12. Pangolin	27.90	27.59	29.75	43.77	48.98	**88.97**	**89.18**	**89.42**	**89.93**	**89.93**	**93.76**	

A group of CoVs with the significant similarity of *N*-proteins are highlighted in grey.

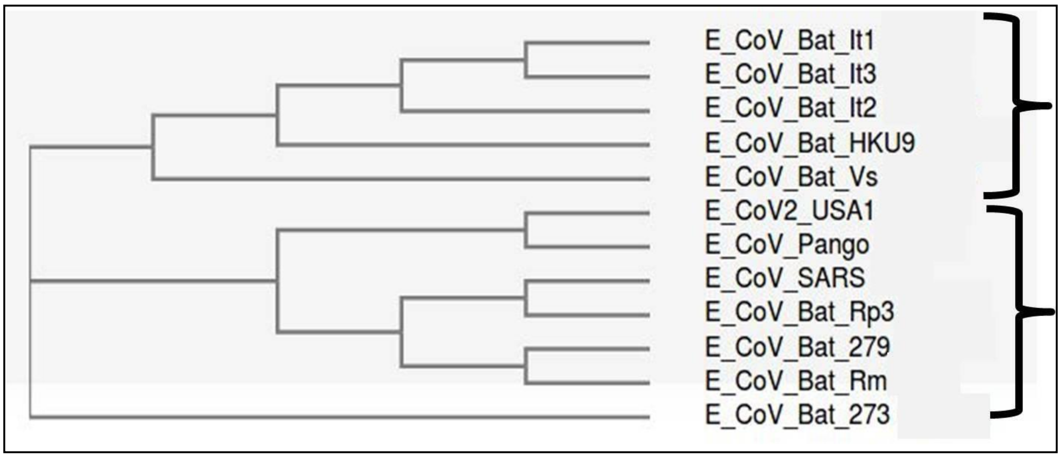


Fig. 1. A phylogenetic tree constructed for on the basis of comparison of E-proteins from 12 CoVs of different species/strains.

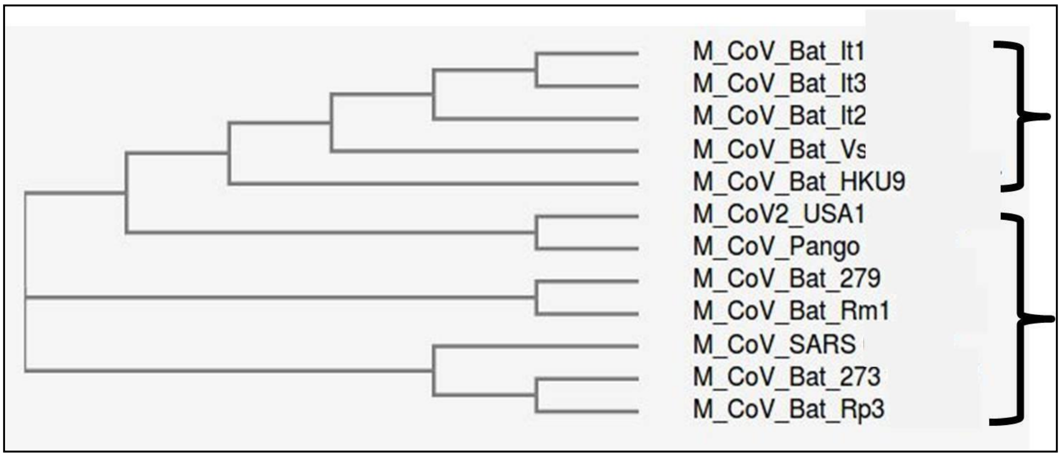


Fig. 2. A phylogenetic tree constructed for on the basis of comparison of M-proteins from 12 CoVs of different species/strains.

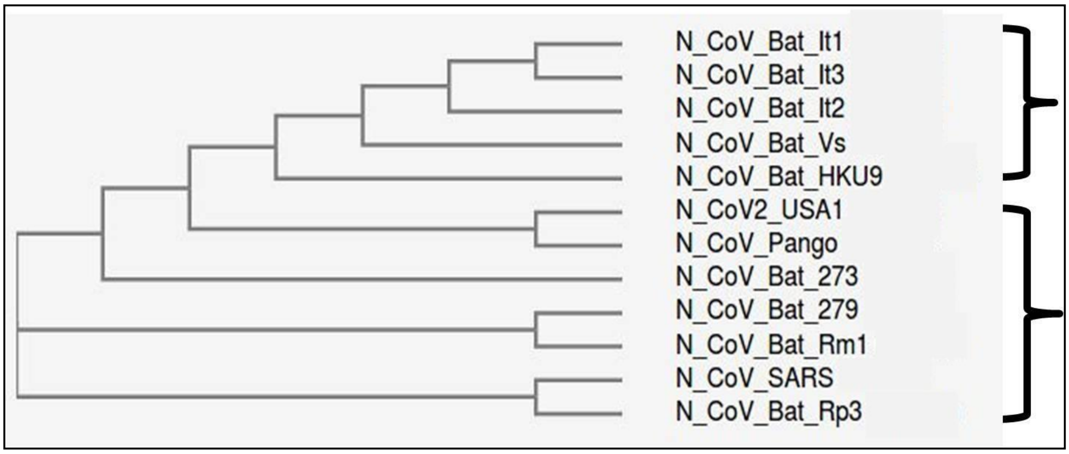


Fig. 3. Phylogenetic tree based on comparison of E-proteins from 12 CoVs of different species/strains.

These findings indicate that at least in bats, various sub-classes of CoVs exist. The different CoV strains in bat have probably been diverged for a long time. Moreover, a significant difference for the similarity level of E, M and N proteins from 4 bat CoV strains is not observed. Taking into account these two facts, for further comparative studies on E, M and N proteins only the following CoVs were selected: SARS-CoV-2, SARS-CoV, pangolin CoV and strain Rp3 of the bat CoV.

First, in each group (E, M and N) of the 4 selected coronaviruses, the proteins were aligned using the Clustal Omega tool. Then, the results of these alignments were analyzed by the MUTAN-2 program. Results of the analysis are summarized in Table 4.

The first remarkable result of these studies is that pangolin CoV is mostly closer to the human SARs-CoV-2 in terms of the inter-species similarity of the E, M and N proteins. Thus, while the similarity between E, M and N proteins from SARS-CoV-2 and pangolin virus is about 100%, 98% and 93%, respectively, these figures for comparisons of SARS-CoV-2 *vs* SARS-CoV and SARS-CoV-2 *vs* bat CoV are 96%, 89% and 89%, respectively. The same picture was observed in the inter-species comparison of S proteins (data not shown). It should be noted that this result is fully consistent with the results of studies recently reported (Lopes et al., 2020; Malaiyan et al., 2020; Zhang et al., 2020).

Compared to proteins M and N, Protein E from human SARS-CoV-2 and SARS-CoV, pangolin CoV, and four strains of bat CoV are almost conserved, although there are also bat CoV strains with significant differences (60-85%; see Table 1). These observations may indicate that while the E proteins are important for the CoV envelope forming, they are not involved in a definition of the host range and pathogenesis of CoVs.

Table 4. The similarity details of the CDS and amino acid sequences of the E, M and N proteins from SARS-COV-2, SARS-CoV, pangolin CoV and bat CoV

	Conservation level: CoV-2 vs Pangolin CoV	**Conservation level: CoV-2 vs SARS CoV**	**Conservation level: CoV-2 vs Bat CoV**
E protein:			
Identities (amino acids)	75 (out of 75), 100.0%	72 (75), 96.0%	72 (75), 96.0%
Identical codons (CDS)	70 (out of 75), 93.33%	65 (75), 86.67%	66/ (75), 88.0%
Synonymous substitutions (CDS)	5 (out of 5), 100.0%	7 (10), 70.0%	6 (9), 66.67%
Non-synonymous substitutions (CDS)	0	3 (10), 30.0%	3 (9), 33.33%
Indels (amino acids)	0	1 (75), 1.33%	1 (75), 1.33%
M protein:			
Identities (amino acids)	218 (222), 98.2%	198 (222), 89.19%	198 (222), 89.19%
Identical codons (CDS)	169 (222), 76.13%	141 (222), 63.51%	3 (193), 1.55%
Synonymous substitutions (CDS)	49 (53), 92.45%	57 (80), 71.25%	59 (82), 71.95%
Non-synonymous substitutions (CDS)	4 (53), 7.55%	23 (80), 28.75%	23 (82), 28.05%
Indels (CDS)	0 (193)	1 (222), 0.45%	1 (222), 0.45%
N protein:			
Identities (amino acids)	391 (419), 93.32%	376 (419), 89.74%	374 (419), 89.26%
Identical codons (CDS)	325 (419), 77.57%	295 (419), 70.41%	291 (419), 69.45%
Synonymous substitutions (CDS)	66 (92), 71.74%	81 (124), 65.32%	83 (128), 64.84%
Non-synonymous substitutions (CDS)	26 (92), 28.26%	43 (124), 34.68%	45 (128), 35.16%
Indels (CDS)	2 (419), 0.48%	3 (419), 0.72%	2 (419), 0.48%

[1]Most significant variations in the amino acid and codon compositions are marked in grey.

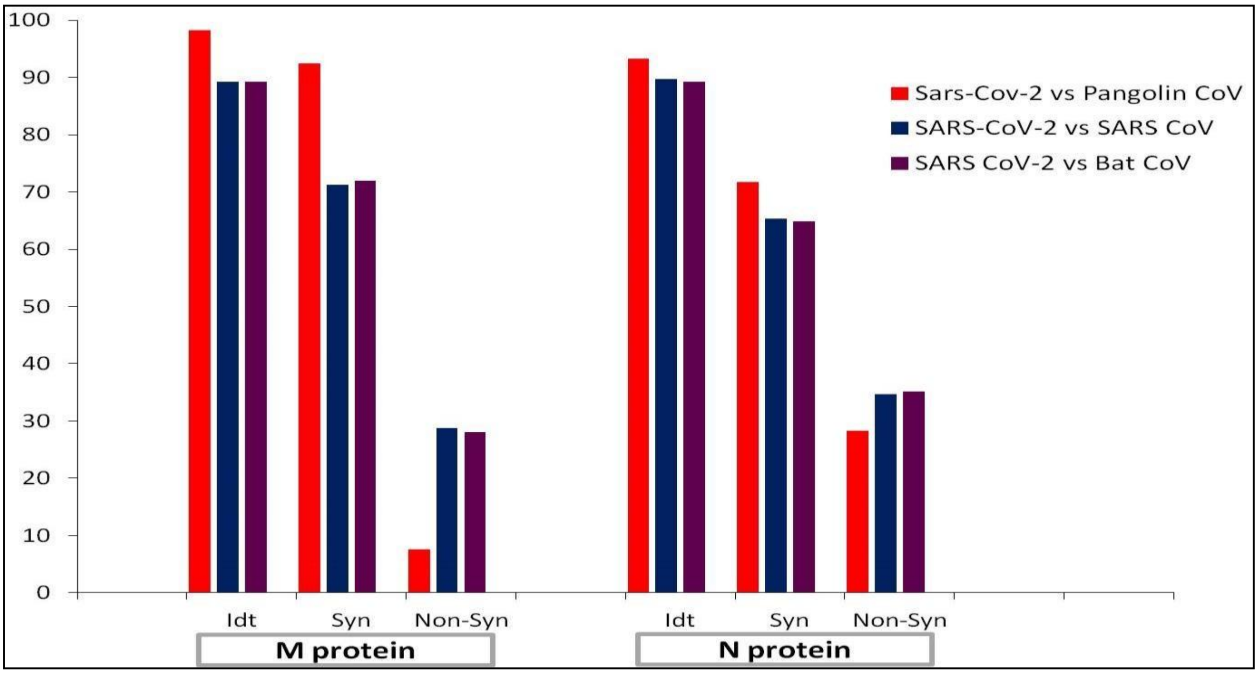


Fig. 4. Graphical representation of differences in identities, synonymous substitutions and nonsynonymous substitutions within M and N proteins between the human SARS-CoV-2 and SARS-CoV, the pangolin CoV and the bat CoV, strain Rp3. Idt – identities, Syn – synonymous mutations, Non-Syn – non-synonymous mutations.

However, the most interesting result was obtained in the comparative studies of the synonymous and non-synonymous substitutions rates in M and N proteins. Thus, out of 53 substitutions in M proteins from the SARS-CoV-2 and pangolin CoV, 49 changes (92.45%) were due to the synonymous substitutions. For the SARS-CoV-2 *vs* ARS-CoV and SARS-CoV *vs* bat CoV comparisons, this rate was significantly lower: 71.25% (57 out of 80) and 71.95% (59 out of 82), respectively. For N proteins, these characteristics were 71.74% (66/92), 65.32% (81/124) and 64.84% (83/128, respectively (Fig. 4; see also Table 4). In particular, these findings suggest that a significant role in almost identity (98.2% similarity) of M proteins in the SARS-CoV-2 and the pangolin CoV belong to the synonymous substitutions. Taking into account these findings and our current knowledge of the key role of M proteins in integration of CoV into the host cell, as well as our recent results on conservation of S proteins in human, pangolin and bat CoVs (unpublished), we suppose that the SARS-CoV and SARS-CoV-2 have some bat CoV and pangolin CoV origin, respectively (Fig. 5).

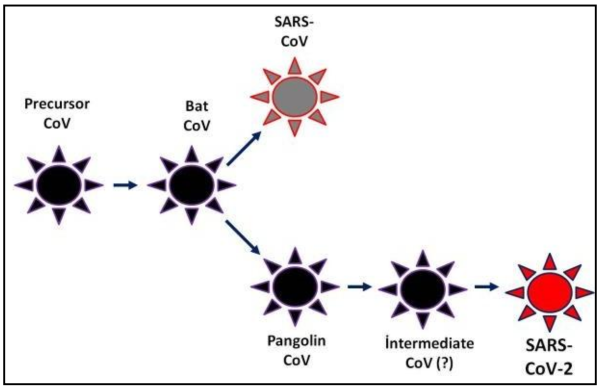


Figure 5. A hypothetical path of evolutionary events resulted in the SARS-CoV-2 and SARS-CoV from a precursor CoV of the unknown origin.

Menachery and colleagues (2015) studied the disease potential for SARS-like virus, SHC014-CoV, from populations of Chinese horseshoe bats. To do this, they developed a chimeric virus and injected it into the mouse backbone. The experimental results showed that the chimeric virus is able to efficiently (i) use orthologues of the SARS receptor for ACE2), (ii) replicate in primary cells of the human respiratory tract, and (iii) achieve *in vitro* titers close to epidemic strains of SARS-CoV. In addition, it has been demonstrated *in vivo* that a recombinant virus can replicate in the lungs of mice with significant pathogenesis using a novel spike protein. These results suggest that full-length recombinant viruses could potentially appear in humans.

It should be noted that our suggestion on the pangolin origin of the SARS-CoV-2 is fully consistent with the results of studies recently reported (Lopes et al., 2020; Malaiyan et al., 2020; Tang et al., 2020; Zhang et al., 2020). But, we understand that the available facts do not preclude a definitive answer to these questions.

CONCLUDING REMARKS

The question on the direct origin of the highly pathogenic SARS-CoV-2 still remains to be answered, although the pangolin origin of this virus seems to be most attractive hypothesis. Moreover, an existence of unknown intermediate organisms in transfer of this virus to humans cannot be excluded.

The question of whether there was human intervention in creation of this virus also remains open. Of course, we are not saying that any malicious people have exposed humanity to a terrible disease like COVID-19 through this coronavirus. However, the possibility of creating a new form of coronavirus to test a scientific idea in a laboratory and then infecting humans as a result of someone's usual negligence, cannot be ruled out.

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SARS-CoV, SARS-CoV-2, kələzin və yarasanın CoV viruslarının E, M və N struktur zülallarının müqayisəli tədqiqi

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Son iyirmi il ərzində bəşəriyyət 3 koronavirus xəstəliyinə düçar olmuşdur, halbuki insan koronavirusları 50 il əvvəl aşkar edilmişdir. 2019-cu ildə kəşf olunan son koronavirus xəstəliyinə (COVID-19) insanın şiddətli kəskin tənəffüs sindromu koronavirusu-2 (SARS-CoV-2) səbəb olur. Sual doğur: son illərdə koronavirusların bu cür aktivləşməsinin səbəbi nə ola bilər? Bu suala cavab vermək üçün, heç olmasa, (1) bu virusların tarixini və mənşəyini və (2) çox asanlıqla və sürətlə sahib hüceyrələrinə daxil olaraq çoxşaxəli ciddi pozğunluqlar törətməsinin molekulyar mexanizmləri aydınlaşdırmaq lazımdır. Bu işdə SARS-CoV-2, SARS, yarasa və kələzin CoV viruslarının E, M və N struktur zülalları müqayisə olunmuşdur. İlk dəfə bu tədqiqatlarda aşkar olunan, ən təəccüblü fakt SARS-CoV-2 və kələz CoV-unun M və N zülallarındakı sinonim əvəzləmələrin nisbi payının digər koronaviruslarala müqayisədə əhəmiyyətli dərəcədə yüksək olmasıdır. Bu fakt SARS-CoV-2-nin yaranması və divergensiya dövrü ilə bağlı yeni suallar doğurur.

Açar sözlər: *Koronavirus, COVID-19, E zülalı, M zülalı, N zülalı, kələz, yarasa, SARS-CoV-2-nin mənşəyi, sinonimik mutasiyalar, sinonimik olmayan mutasiyalar*

Сравнительные исследования структурных белков E, M и N SARS-CoV, SARS-CoV-2, CoV панголина и летучей мыши

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В течение последних двух десятилетий человечество страдает от 3 коронавирусных заболеваний, хотя коронавирусы человека были обнаружены более 50 лет назад. Последнее коронавирусное заболевание, обнаруженное в 2019 году (COVID-19), вызвано коронавирусом 2 тяжелого острого респираторного синдрома человека (SARS-CoV-2). Возникает вопрос: в чем может быть причина такой активации коронавирусов в последние годы? Чтобы ответить на этот вопрос, по крайней мере, необходимо выяснить (1) историю и происхождение этих вирусов, (2) изучить молекулярные механизмы их очень легкого и быстрого проникновения в клетки-хозяева, влекущего за собой многогранные серьезные нарушения. В этом исследовании мы сравнили структурные белки E, M и N из вирусов SARS-CoV-2, SARS, CoV летучих мышей и панголинов. Наиболее поразительный факт, впервые обнаруженный в этом исследовании, заключается в том, что относительная доля синонимичных скоростей замен в M и N белках SARS-CoV-2, и CoV панголина значительно выше, чем соответствующие характеристики для других изученных CoV. Это факт ставит несколько интригующих вопросов о возникновении и продолжительности дивергенции SARS-CoV-2.

Ключевые слова: *Коронавирус, COVID-19, E-белок, M-белок, N-белок, ящер, летучая мышь, происхождение SARS-CoV-2, синонимичные мутации, несинонимичные мутации*

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Co-infections in COVID-19 patients and the importance of microbial diagnosis for disease management

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Patients infected with respiratory viral infections especially new coronavirus disease (COVID-19) are most susceptible to co-infections which in turn, increases the severity of disease and mortalities. Therefore, antibiotic agents should be applied for the treatment of bacterial co-infection and super-infections. On the other side, all guidelines for COVID-19 clearly mention that improper use of antibiotics, especially the combination of broad-spectrum antibiotic agents, should be avoided. Because the use of broad-spectrum antibiotics for a long time and in the combination of several other agents not only shows no effect on the recovery of the disease but also may lead to potentially fatal secondary superinfections and induce resistance in the normal bacterial population. Currently, due to the unavoidable use of antibiotics among patients with COVID-19 who are admitted to intensive care units, cultivation-based methods for isolating and detecting bacteria are less sensitive in the management of the disease. Hence, the use of culture-independent methods that can detect a wide range of potential pathogens and antimicrobial resistance is important, especially for screening and treatment follow-ups. So, culture-independent techniques such as whole-genome metagenomics can be used to identify monomicrobial or mixed infections without selecting the previous target. Whole-genome metagenomics can provide valuable and useful information about pathogens that cause co-infections and antimicrobial resistance in hospital settings, especially in the intensive care units. Therefore, these studies can have a valuable aid in the management of antibiotic administration and subsequent targeted treatment of infections.

***Keywords:** COVID-19, bacterial infection, antimicrobial, whole-genome metagenomics*

INTRODUCTION

Viral infections of the respirstory system makes patients vulnearable to coinfections and superinfections and leading to high levels of mortalities and severity of disease (Cornbleet et al., 2002). Poor treatment outcomes of 2009 H1N1 influenzae pandemic was also attributed to the opportunistic coinfections (Ardron et al., 1994). Zhou et al., showed that 50% of Covid-19 patients who die have an additional bacterial superinfections (Zhou et al., 2020). Emerging Covid-19 pandemy has shown that health and diagnostic laboratory infrastructures needs to be revised in many of countries all over the world, especially in the context of rapid identification of organisms and management of antibiotics applications, as well as the control and management of additional bacterial or fungal infections in critical infectious conditins (Cox et al., 2020; MacIntyre et al., 2018). In fact, various studies have reported low bacterial or fungal infections in Covid-19 patients (Phlan et al., 2020; Zhou et al., 2020; Yu et al., 2020;

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Huang et al., 2020; Arentz et al., 2020; Chen et al., 2020; Goyal et al., 2020; Chen et al., 2020), but in other studies, high opportunistic microbial infections such as bacterial pneumonia, and respiratory viruses have been reported in critically ill Covid-19 patients, which attributed to the samplig methods and number of samplings (Bachmann et al., 2018; Gautret et al., 2020). However, in other studies, the rate of coinfection with different pathogens were reported to be from 50 to 94% of patients, and bacteria including *S. pneumonia*, *K.pneumoniae* and *H.influenzae* were reported as dominant coinfectious agents in Covid-19 patients (Bates et al., 1992; De et al., 2016). But, in general, there is still no comprehensive information about the coinfections associated with Covid-19 infections and the types of pathogens involved in the pulmonary system, as well as their impact on disease severity and treatment outcome (De et al., 2016). Therefore, it is necessary for laboratory specialists with the help of clinicians in such cases to cooperate in order to accurately identify the organisms responsible for superinfection or coinfections. In this context, a combination of phenotypic routine, or new molecular and early biomarkers were suggested in the literature. Certainly, according to the treatment results obtained for Covid-19 disease, antibiotic treatments have caused many complications and side effects for patients due to their empirical nature and the use of a wide range of drugs.

Do Covid-19 patients have microbial coinfections, or is it acquired as a superinfection in the hospital?

In many studies, about 6 to 8% of microbial coinfection has been reported in Covid-19 patients. In these patients, it is not clear whether these rates are related to nosocomial infections or not (Arentz et al., 2020; Chen et al., 2020; Huang et al., 2020; Phlan et al., 2020; Yu e tal., 2020; Zhou et al., 2020). But a study in China reported 15 percent of nosocomial infections in Covid-19 patients. In this study 27 out of 28 nosocomial infections was died in hospital which indicates that coinfections could complicate and affect the mortalities of patients (Phlan et al., 2020).

There is no detailed report on the type of genus and microbial species involved in the coinfection or nosocomial infection after admission of patients in hospitals based on standard identification methods. And in different studies poly or mono-microbial infections composed of *A.baumannii*, *K.pneumo-niae*, *E.cloacae*, *S.pneumoniae*, *S.aureus*, *M.pneumoniae*, *C.pneumonia*, *L.pneumophila*; *A.fumigatus* and Candida species; and viruses such as influenza, coronavirus, rhinovirus/enterovirus, parainfluenza, metapneumovirus, influenza B virus, and human immunodeficiency virus have been reported from sputum or tracheal aspirate samples (Bates et al., 1992; Chen et al., 2020; De et al., 2016; Gautret et al., 2020; Goyal et al., 2020; Wang et al., 2020). Sometimes no organism has been reported in microbial studies of patient samples (Bonadio et al., 1992; Emmanuel et al., 2020).

In a large review of 1007 abstracts, it was found that 62 out of 806 cases of Covid-19 patients had bacterial or fungal infections at the time of admission (about 8%), while subsequent studies found that most Patients (about 72%) were prescribed broad-spectrum antibiotics (Cox et al., 2020). Of course, this could be due to the fact that the majority of patients develop secondary infections after hospitalization, which can be considered for better patient management.

Empirical versus antibiogram based antibiotic prescription in Covid-19 patients

Opportunistic or coinfections are inevitable in viral pulmonary diseases, therefore, antibiotics should be used to help treating such diseases. But usually clinicians, prescribing broad-spectrum antibiotics mostly as empirically. Which may have problems for patients.

On the other hand, bacterial/fungal/viral coinfections appears to be low in Covid-19 patients. But, broad-spectrum antibiotics have been used in most studies, especially in the later stages of the disease. For example, in Cao et al. studies, 101 out of 102 (99%) patients have been treated with quinolones, cephalosporines, and carbapenems (Cao et al., 2020). In a study by Guan et al., 637(58%) and 31(3%) out of 1099 patients received antibacterial and antifungals, respectively (Guan et al., 2020).

However, coinfections appear to be less reported in studies. But, the positive effect of prescribing antibiotics in patients shows that in order to prevent the side effects of drugs, opportunistic infectious organisms should be well identified in the laboratory

and antibiogram tests should be performed to determine the correct antibiotic for treatments (Ardron et al., 1994; Kuppermann et al., 1999).

Neccessity on the microbial identification in the Covid-19 patients

Based on the explanations given and the results of various studies, it is clear that coinfections, both as a actual concomitantly microbial or as a hospital-acquired superinfections, are seen in high rates in Covid-19 and other coronavirus patients (Bates et al., 1992; Cornbleet et al., 2002; Touzard et al., 2013; De et al., 2016; Chih-Chang Lai et al., 2020). On the other hand, the widespread use of broad-spectrum antibiotics in the treatment of these infections suggests that patients are forcibly infected by other opportunistic organisms (Timpthy et al., 2020; Zhou et al., 2020). In order to increase the success of treatment, concomitant bacterial/fungal infectious agents must be identified accurately to the genus and even species levels.

However, coinfections in some reported cases, such as *M.pneumoniae* with Covid-19, has not been associated with disease severity. But, at the same time the outcome of the complications of coinfections or superinfections with the virus is still unknown (Gautret et al., 2020). And as a fact, accumulating data shows that respiratory microbial coinfections incresing the risk of disease severity in humans (De et al., 2016).

In fact, the main problem in downplaying the role of identifying organisms in the treatment of Covid-19 patients is that physicians generally prefer emprical treatment with broad-spectrum antibiotics using clinical signs. Therefore, in some cases, after empirical treatments, death occures in the patients as a result.For this reason, in order to help properly manage the treatment of Covid-19 patients, it is suggested that microbial agents of coinfections or superinfections, should be detected to genus level in the patient samples, and effective and selective antibiotics should be determined for proper treatment of patients by standard antibiogram tests.

Culture based versus non-culture based methods

More than 24 different organisms have been reported from Covid-19 patients sputum or bronchial washing samples as coinfections and superinfections (Bates et al., 1992; De et al., 2016), which complicating the treatment and management of disease, and this emphasizes the accurate isolation and identification of microbes from patient samples. In some cases it is claimed that viruses such influenza A as a most common coinfecting viruses could cause a false-negative results in real-time RT-PCR tests in Covid-19 patients. Even it has been mentioned that laboratory and imaging results alone cannot help for differentiation of concomitant microbial infections from Covid-19 infections. So, in fact, some kind of identification of coinfections or superinfections should be used for analysis of patients samples. It is suggested that new multiplex molecular panels based on syndromic patterns with incorporation of Covid-19 virus probabely help to the early and correct detection of coinfecting organisms (Bates et al., 1992). Therefore, it seems that even routine methods of identification based on microbial culture and phenotypic tests can not be useful in differentiating the main causes of coinfections, so it is better to use new molecular methods, including diagnostic metagenomics, Whole genome sequencing (WGS), next-generation sequencing for accurate identification Infectious organisms should be used so that patients can be treated more accurately (Zarkesh et al., 2015; Paliogiannis et al., 2020; Zhihua et al., 2020).

DISCUSSION AND CONCLUSIONS

According to various studies, although primary microbial coinfection appears to be low in Covid-19 patients, the use of broad-spectrum antibiotics in the majority of patients means that like most human infections these patients are also attacked by opportunistic bacteria. But, certanly empirical therapy with broad-spectrum antibiotics is not a right and corrects method in management of patients. At present, due to the urgency of the treatment of critically ill patients, we can not give a definite opinion on the widespread use of antibiotics, or the prescription of broad-spectrum antibiotics. Meanwhile, at least we know that due to the high dose of drugs used and the severe involvement of the immune system in Covid-19 infections and phenomena such as cytokine storms, and the possibility of drug resistance induction in normal flora bacteria, many problems are possible as threatening patients under antibiotics. Therefore, the global scientific community should think of a consensus on identifying the causes of coinfection or superinfections in Covid-19 patients and highlight the role of microbiological laboratories.

On the other hand, there are no reports of side effects or complications from the hasty use of broad-spectrum antibiotics in coronavirus patients. Some drug groups, such as macrolides, also have antiviral effects, so their effect on coronavirus patients should be compared with other broad-spectrum antibiotics, to define that if their effect on disease control is different from other antibiotics (Xianjouan et al., 2019).

Early detetion biomarkers, such as calcitonin, band cells, increased mean total white blood cell (WBC) and absolute band counts (ABC), and high IL-6, interleukin-10, C-reactive protein, and D-dimer levels in critical ill patients, can also be used to differentiate between types of microbial infections(bacterial or viral) and to determine the need for antibiotics as a more non-invasive methods (Yap et al., 2004; Pallen et al., 2014; Dekker et al., 2018; Arabi et al., 2019; Cao et al., 2020; Guan et al., 2020; Kim et al., 2020; Liang et al., 2020; Wu et al., 2020). In some studies, this has been addressed in more detail. For example, one study found that lymphopenia was more severe in men than women, and that in patients with critical symptoms the tumor necrosis factor alpha was lower than the severe or mild patients. They reported about 24% coinfection with other respiratory pathogens in critical and severe cases,and suggested that coinfection, lymphocyte count and d-dimer have relation with severity of Covid-19 infections (Dekker et al., 2018). In other study, analysis indicate that bilirubin level was very higher in severe COVID-19 patients (Meier et al., 2019). This suggests that early detection criteria can also be used to differentiate between clinical status and the severity of disease, as well as treatment management.

Another important issue in the need for careful microbiological examination of samples of Covid-19 patients and identification of microbes involved in coinfections is that inappropriate and incorrect treatments can affect the outcome of the patient's recovery.

The bottom line is that, in order to reduce the side effects of broad-spectrum antibiotics in emergency patients, we must first be able to detect nosocomial infections in hospitalized patients through laboratory routine or molecular new methods. Then, in order to target antibiotic therapies, we need to use of standard antibiograms to determine the correct antibiotics for treatment.

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COVID-19 xəstələrində ko-infeksiyalar və xəstəliklərin idarə olunmasında mikrobioloji diaqnozun əhəmiyyəti

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Tənəffüs yoluxucu viral infeksiyalara, xüsusən də yeni koronavirus xəstəliyinə (COVID-19) yoluxmuş xəstələr ən çox yoluxucu birgə infeksiyalara həssasdırlar və bu da xəstəliklərin və ölümlərin ağırlığını artırır. Bu səbəbdən antibiotik agentləri bakterial birgə infeksiyalar və superinfeksiyaların müalicəsi üçün tətbiq olunmalıdır. Digər tərəfdən, COVID-19 üçün bütün təlimatlarda antibiotiklərin, xüsusən də geniş spektrli antibiotik agentlərinin birləşməsinin düzgün istifadə edilməməsi lazım olduğu açıq şəkildə qeyd edilir. Çünki geniş spektrli antibiotiklərin uzun müddət və bir sıra digər maddələrin birləşməsində istifadəsi nəinki xəstəliyin bərpa olunmasına təsir göstərir, həm də normal bakterial populyasiyada ölümcül potensial ikincil superinfeksiyalara və müqavimətə səbəb ola bilər. Hal-hazırda reanimasiya şöbələrinə müraciət edən COVID-19 xəstələri arasında qaçılmaz antibiotik istifadəsi səbəbindən bakteriyaların təcrid edilməsi və aşkarlanması üçün becərmə əsaslı üsullar xəstəliyin idarə edilməsində daha az həssasdır. Beləliklə, geniş bir potensial patogen və antimikrob müqavimət aşkar edə bilən kulturadan asılı olmayan metodların istifadəsi, xüsusən də müayinə və müalicə təqibləri üçün vacibdir. Beləliklə, əvvəlki hədəfi seçmədən monomikrob və ya qarışıq infeksiyaları təyin etmək üçün bütöv genom metagenomikası kimi kulturadan asılı olmayan üsullardan istifadə edilə bilər. Bütün genom metagenomikası, xüsusilə reanimasiya şöbələrində xəstəxana şəraitində birgə infeksiya və antimikrob müqavimətə səbəb olan patogenlər haqqında dəyərli və faydalı məlumatlar verə bilər. Bu səbəbdən, bu tədqiqatlar antibiotik verilməsinin və infeksiyaların sonrakı hədəflənmiş müalicəsinin idarə edilməsində dəyərli bir köməkçi ola bilər.

Açar sözlər: *COVID-19, bakterial infeksiya, antimikrob, bütün genom metagenomikası*

Сопутствующие инфекции у пациентов с COVID-19 и важность микробной диагностики для лечения заболеваний

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Пациенты, инфицированные респираторными вирусными инфекциями, особенно новым коронавирусным заболеванием (COVID-19), наиболее восприимчивы к сопутствующим инфекциям, что, в свою очередь, увеличивает тяжесть заболевания и смертность. Следовательно, для лечения бактериальной инфекции, сопутствующей суперинфекциям, следует применять антибиотики. С другой стороны, во всех рекомендациях по COVID-19 четко упоминается, что следует избегать неправильного использования антибиотиков, особенно комбинации антибиотиков широкого

спектра действия. Потому что использование антибиотиков широкого спектра действия в течение длительного времени и в сочетании с несколькими другими агентами не только не способствует выздоровлению, но и может привести к потенциально смертельным, вторичным суперинфекциям и индуцированию устойчивости у нормальной популяции бактерий. В настоящее время из-за неизбежного использования антибиотиков среди пациентов с COVID-19, которые поступают в отделения интенсивной терапии, методы выделения и обнаружения бактерий, основанные на культивировании, менее чувствительны для лечения болезни. Следовательно, важно использовать методы, не зависящие от посева, которые могут определять широкий спектр потенциальных патогенов и устойчивость к противомикробным препаратам, особенно для скрининга и последующего лечения. Таким образом, независимые от культуры методы, такие как метагеномика всего генома, могут использоваться для выявления мономикробных или смешанных инфекций без выбора предыдущей мишени. Полногеномная метагеномика может предоставить ценную и полезную информацию о патогенах, вызывающих сопутствующие инфекции и устойчивость к противомикробным препаратам в больничных условиях, особенно в отделениях интенсивной терапии. Следовательно, эти исследования могут оказать ценную помощь в управлении назначением антибиотиков и последующем целенаправленном лечении инфекций.

Ключевые слова: *COVID-19, бактериальная инфекция, противомикробный, полногеномная метагеномика*

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Immune strengthener plants in Azerbaijan flora

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The main goal of the work was to study immunomodulating and strengthening plants in the flora of Azerbaijan. The frequency of use (Ui) and the value of plant species relative to each other due to their immunomodulatory properties have been evaluated by new methods. The total amount of data submitted by the person providing for each specific species is marked as Ni. The result of the ratio of immunomodulators have been shown in the diagram: the highest IQ (assessment of use) was found for *Allium* sp. and *Thymus* sp. (0.90-0.88), the next places are occupied by *Rosa* sp. (0.83), *Salvia limbata* (0.81), *Altheae officinalis* (0.80), *Glycyrrhiza glabra* (0.78), *Mathricaria* sp. (0.77), *Humulus lupulus* (0.74), *Mentha* sp. (0.60), *Sitrus* ssp. (0.27), *Capsella burssa pastoris* (0.21), *Rumex* sp. (0.18), *Sambucus nigra* and *Helichrysum plicatum* (each by 0.15), *Daucus carota*, *Urtica dioica* and *Asparagus officinalis* (each by 0.10, the lowest IQ).

Keywords: *Ethnobotany, anti-viral plants, immune-reducing agents*

INTODUCTION

The benefits of plants for physical and mental health have long been known to mankind. Influenza viruses and other similar viruses have been plaguing to mankind for centuries (bu ümumi bəşəriyyətə məlum olan cümlədi bir yerdən götürmədim). Those who have a weak immune system suffer more, and those who are strong survive. Herbs have been used as a natural remedy for various diseases, including infections for centuries. Herbal treatment is more important than chemical drugs in the fight against diseases in modern alternative medicine (Munir et al., 2018).

Immunological protection is conventionally classified into 2 categories: congenital and acquired (Beck et al., 1996). Congenital or non-specific (natural, non-adaptive) immune system is the initial stage of the body's protection against biological aggression. Components of the innate immune system are readily available in humans and animals at moments of birth, and their fight against the antigen occurs quickly, without the need for any preparation. An adaptive or acquired immune system (specific) is the next stage of defense. The acquired immune system is formed by lymphocytes during the individual development of the body's ontogeny (Parkin et al., 2001). These systems differs from the innate immune system by high specificity and memory. Specific immunological response is created against each antigen that enters the body in this case. The encounter with the antigen is stored in the memory of the immune system, and stronger defense is formed against under second encounter with the antigen. Cells are able to distinguish a group of foreign molecules in the innate immune system in contrast, not every antigen molecule.

Immune preparations are aimed at producing of high levels interferon based on human blood analyses. The interferon protein both prevents the multiplication of viruses and acts as an immunostimulant (De Andrea et all., 2002). So clearly shows the consequences of improper use of immune drugs and stimulants. On the other hand, no matter how mild the natural remedies, keep in

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mind that these are also medicines. There is a greater need for immune treatment (both herbal and chemical) in the autumn and winter months. Onion (*Allium* sp.) and thyme (*Thymus* sp.) are the most widely used antiviral plants among local communities (Ibadullayeva et al., 2015), these plants are collected and used by local communities as antiviral agents.

We self must to help our immune systems by stimulants, for example.

The most popular herbal immunomodulators are ginseng, eleutherococcus, echinacea, aloe, kalanchoe, tarragon and baby mushrooms. However, plants distributed in the flora of Azerbaijan have also been introduced into production as immuno-modulators (Kakhramanova et al., 2017)

The main goal of research is not only to study ethnopharmacological activity, but also to determine the biologically active plants for their further use (Martin, 1995).

MATERIAL AND METHODS

Collection and identification of plants. Medicinal plants were collected from the study area with the help of herbalists and deposited to the Herbarium at the Institute of Botany (BAK). These plants were pressed and poisoned with 1% $HgCl_2$ solution, mounted at herbarium sheet and identified (Ibadullayeva, 2020; Askerov, 2016; Flora of Azerbaijan, 1950-1961).

Ethnomedicinal study was conducted in the period of March-June (2010-2020) and August–December (2010-2019). A preliminary visit of district administration that has authority to register the local herbalists to get the information about the local herbalistsas key informants was conducted. The rural community dependent upon the traditional use of indigenous plants for a number of diseases due to low income and far-flung health facilities.

Data analysis. Data collected through various field surveys was analyzed through statistical software SPSS version 9.00 (Nadeem et al., 2015). Multiresponse of families was calculated with plants habits, preparations and applications. The data was also represented using the percentages and proportions. Each plant species reported by indigenous informant was counted by or frequency of citation (FC).

The relative importance of plant species was evaluated by calculating its use value as described by Phillips and Gentry (1993). Use value was calculated according to the the formula $UVi=\Sigma Ui/Ni$, where number of use report(s) cited by each informer for specific plant species i is represented by Ui and total number of informs interviewed for specific plant species i is represented by Ni (table 1).

Data was collected through rapid appraisal approach (RAA) by direct interact with local people and observations during the visits. Group meetings were conducted with people having adequate knowledge about indigenous plants and individual meeting were arranged with herbalists to countercheck the data. During the course of study 600 informants including 110 herbalists were interviewed from the district of Azerbaijan.

RESULTS AND DISCUSSION

The following conclusions are drawn from the data collected on plant use. *Allium cepa* was the predominant plant which exhibited highest used value (0.90). It attributed the important recognition with local healers and efficient healing power against different ailments. Followed by *A.cepa*, *Thymus* sp. (0.88) exhibited higher UV while *Rosa* sp. reflected 0.83 UV followed by *Allium sativum* (0.82), *Salvia* sp. (0.81), *Althea officinalis* (0.80), *Glycyrrhiza glabra* (0,78), *Mathricaria* sp. (0.77), *Mentha* sp. (0.60), *Humulus lupulus* (0.74), *Daucus carota, Urtica dioica* and *Asparagus officinalis* (0.10) showed lowest UV and upshiftin UV was observed as *Sambucus nigra* (0.15), *Helichrysum plicatum* (0.15), *Rumex* sp. (0.18), *Capsella burssa pastoris* (0.21) and *Citrus sp.* (0.27). It is observed that the plant with high uses value were found frequently in the study area and the plant with low used value were some what fewer in availability in the study area. The diagram below shows the types identified (Fig. 1).

Some plants are widely used in the treatment of other diseases, including antivirals as have shown studies. Using antipyretic, anti-inflam-matory, antiseptic, antitussive, antispasmodic and general strengthening herbs are necessary in diseases of the respiratory system.

Table 1. Data about number of diseases and plants used for their prevention represented by interviewed people

Local and Latin names of plant genera	Number of diseases	Local and Latin names of plant genera	Number of diseases	Local and Latin names of plant genera	Number of diseases
Sarımsaq – *Allium sativum*	48	Əvəlik – *Rumex confertus*	12	Kəndəlaş – *Sambucus nigra*	7
İtburnu – *Rosa canina*	21	Havuc – *Pastinaca umbrosa*	11	Qarğıdalı – *Zea mays*	7
Kəklikotu – *Thymus kotschyanus*	45	Mərəcöyüd – *Asparagus officinalis*	9	*Solmazçiçək – Helichrysum plicatum*	7
Adaçayı – *Salvia limbata*	19	Zeytun – *Olea europaea*	10	Atılbatıl – *Zosima orientalis*	6
Gulxətmi – *Althea officinalis*	18	Uşqun – *Rhehum ribes*	9	İydə – *Elaeagnus angustifolia*	6
Cobanyastığı – *Mathricaria chamomilla*	16	Ardıç – *Juniperus communis*	9	Qudrət narı – *Momordica charantia*	5
Nanə - *Mentha piperita*	15	Mayaotu – *Humulus lupulus*	*10*	Zirə – *Carum carvi*	6
Amarant – *Amarantus viridis*	14	Dəfnə - *Laurus nobilis*	9	Biyan – *Glycyrrhiza glabra*	6
Baldırqan – *Heracleum trachyloma*	9	Əməköməci – *Malva sylvestris*	9	Quşəppəyi – *Capsella bursa-pastoris*	7
Heyva – *Cydonia oblonga*	8	Gicitkan – *Urtica dioica*	13	Alca – *Prunus divaricata*	5
Soğan – *Allium* sepa	8	Çörəkotu – *Nigella sativa*	13	Yemişan – *Crataegus pentagyna*	6
Ceviz – *Juglans nigra*	7	Yer kökü – *Daucus carota*	13	Səhləb – *Dactylorhiza maculata*	4
Dağtərxunu - *Tanacetum vulgare*	8	Ökuzboğan – *Bupleurum falcatum*	6	Qantəpər – *Cephalaria giganteae*	2
Üzərlik – *Peganium harmala*	5	İspanaq – *Spinacia okeraceae*	1	Böyürtkən – *Rubus caesius*	4
Atşabalıdı – *Aesculus glabra*	3	Ərik – *Prunus armeniaca*	3	Lavanda – *Lavandula dentata*	6
Daziotu – *Hypericum perforatum*	9	Şüyüd – *Anethum graveolens*	13	CaytikanI – *Hippophae rhamnoides*	3
Çaşır – *Prongos acaulis*	3	Zirinc – *Berberis vulgaris*	4	Ayrıqotu – *Agropyron crstatum*	3
Əncir – *Ficus carica*	3	Badam – *Prunus dulcis*	3	Limon – *Citrus linon*	5
Bibər – *Capsicum annuum*	3	Qovaq – *Populus gracilis*	3	Küstüşam – *Bronya alba*	3
Cəfəri-*Petroselenum crispum*	3	Quşüzümü – *Solanum nigra*	3	Nar – *Punica granatum*	4
Kartof – *Solanum tuberosum*	3	Sumaq – *Rhus lancea*	3	At pıtrağı – *Arctium lappa*	2
Arpa – *Hordeum vulgare*	2	Çovdar – *Secale cereale*	2	Toppuztikan – *Echinops latifolius*	2
Buğda – *Triticum aestivum*	2	Çiriş out – *Eremurus speciosus*	3	Innab – *Ziziphus jujuba*	2
Cökə - *Tilia cordata*	2	Qara turp – *Raphanus sativus*	2	Qalxansız – *Athyrium filix-femina*	2
Qatırquyruğu – *Equsetum arvense*	2	Dəvətikanı – *Alhagi pseudalhagi*	1	Sığırquyruğu – *Verbascum denisiflorum*	6
Razyana – *Foenicuum vulgare*	2	Keçi buynuzu – *Ceratonia siliqua*	2	Qaracöhrə – *Taxus baccata*	2
Xiyar – *Cucumis sativus*	2	Qaytarma - *Potentilla erecta*	2	Xoruzgülü – *Primula officinalis*	2
Şahtərə – *Fumaria officinalis*	2	Xashxash – *Papaver hybridum*	1	Andız – *Inula helena*	8
Cincilim – *Stellaria media*	1	Dovşankələmi – *Crassula*	1	Dəliçətənə - *Datisca glabra*	1
Bədrənc – *Melisa officinalis*	1	Qovaq – *Populus*	1	Dağdağan – *Celtiscaucasica*	1
Gulabətin – *Pulsatilla*	1	Armud – *Pyrus*	1	Qarayonca – *Medicago officinalis*	1
Qumluca – *Arenaria serpyllifolia*	1	Ayıdöşəyi – *Dryopteris filix mass*	2	Gəvən – *Astragalus dasyanthus*	1
Tərə – *Chenopodium album*	1	Murdarca – *Rhamnus pallasii*	1	Pomidor – *Tomate sativa*	1
Quşarmudu – *Sorbus latifolia*	1	Dagkişnişi – *Bifora radians*	1	Alma – *Malus coronaria*	1
Əzgil – *Mespilus germanica*	2	Üçrəng bənovşə – *Viola tricolor*	1	Subibəri – *Persicaria hidropiper*	1
Yabanı kök – *Daucus carota*	1	Xardal – *Sinapis alba*	1	Bistort-*Bistorta major*	1
Yalançı cirə – *Pimpinella*	1	Qərənfil – *Dianthus orientalis*	1	Gilas – *Prunus avium*	1
Kişniş – *Coriandrum*	1	Pişikotu – *Valerianus officinalis*	1	Yulqun – *Tamarix ramosissima*	1
Tut – *Morus nigrum*	1	Yolotu – *Polygonum avicu-lare*	1	Söyud – *Salix nigra*	1
Küncüt – *Sesamum indicum*	1	Quzu qulağı – *Oxalis asetosella*	1	Portağal – *Citrus sinensis*	1

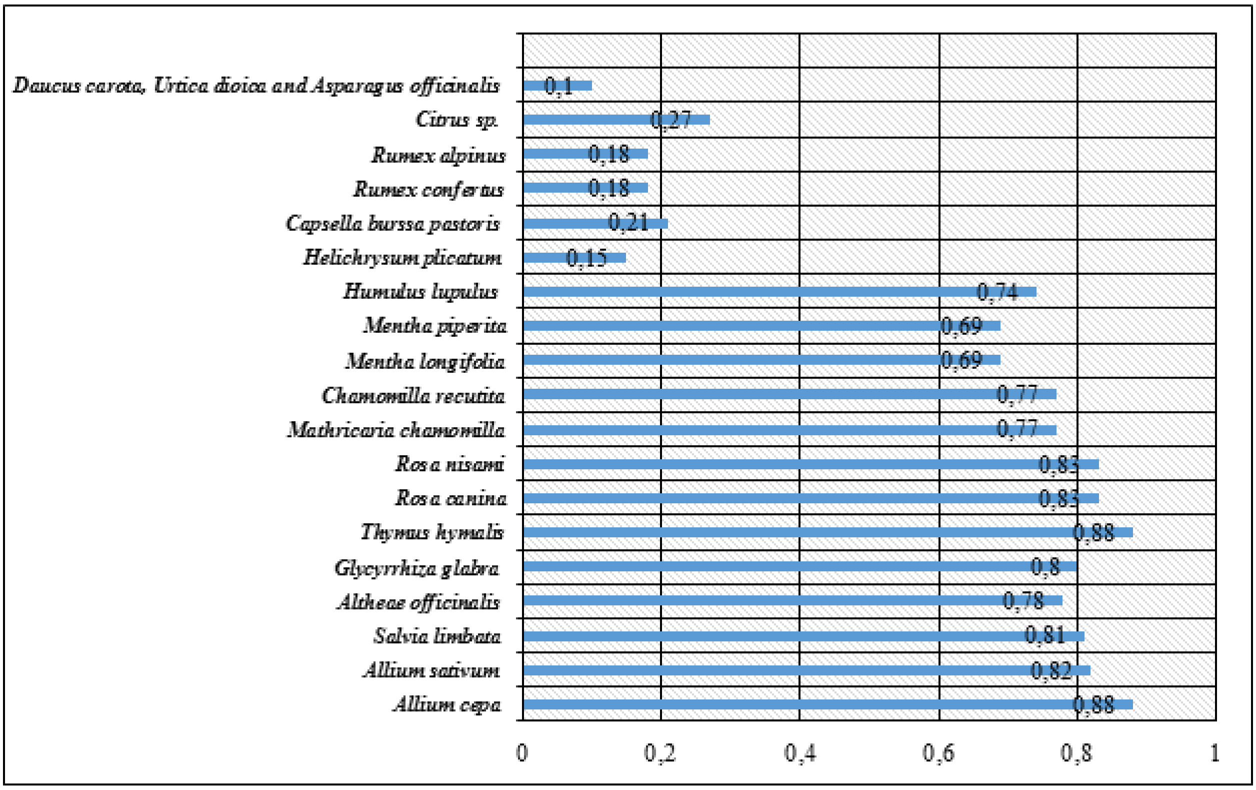


Fig. 1. Usevalue of frequently used plants.

In view of all this, the immunosuppressive properties of some plant mixes have been determined. For the first time, a phyto-collection has been prepared to be produced in the pharmaceutical industry.

Plants collected according data of field surveys and observations conducted by botanists, lojmans, biology teachers, pharmacists and other local communities, generally local experts in remote areas of Azerbaijan for many years.

The data presented below are the results of research conducted in all surveyed districts (more than 50) for 2010-2020 years. The immuno-modulatory phyto-collection was tested in an outpatient setting: blood samples were taken from a 10-year-old sick child and a 42-year-old sick man with weak immune systems, and the phyto-herbal syrup prepared 3 times a day for 3 weeks was given to a sick child and phyto-tea to a sick man for 3 weeks. The analysis of blood taken from patients after treatment was in full compliance with the norm. Increased protective capacity of the body. Unique effect on the rapid recovery of complex colds, flu, bronchitis, angina, pneumonia, bronchial asthma and infections have revealed. Following plant phytonutrients as a medicine is recommended as a restorative and protective of the immune system, especially for those who live in unfavorable climatic and environmental conditions. The phyto-collection contains the surface part of *Echinacea purpureae, Hypericum perfaratum* herb, *Glycyrrhiza glabra* roots, *Rosa canina* fruits, *Salvia limbata* grass, *Rosmarinus officinalis* grass and *Rubus idaeus* fruits in 1:1 ratio.

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Azərbaycan florasının immunmöhkəmləndirici bitkiləri

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İşin əsas məqsədi Azərbaycan florasında olan immunbərpaedici və möhkəmləndirici bitkilərin öyrənilməsi olmuşdur. Bitki növlərinin bir-birinə nisbətən immunbərpaedici xüsusiyyətinə görə istifadə tezliyi (Ui) və əhəmiyyəti, yeni metodlarla qiymətləndirilmişdir. Hər bir məlumat verənin konkret göstərilən bitki növləri üçün istifadəsi haqqında məlumatların ümumi sayı N_i ilə təmsil olunurmuşdur. Nəticədə immunbərpaedicilərin nisbəti diaqramda göstərilmişdir: *Allium* sp. və *Thymus* sp. (0,90-0,88) daha yüksək İQ (istifadə qiymətləndirilməsi) nümayiş etdirmişdir. *Rosa* sp. (0,83), *Salvia limbata* (0,81), *Altheae officinalis* (0,80), *Glycyrrhiza glabra* (0,78), *Mathricaria* sp. (0,77), *Humulus lupulus* (0,74), *Mentha* sp. (0,60), *Sitrus* ssp. (0,27), *Capsella burssa pastoris* (0,21), *Rumex* sp. (0,18), *Sambucus nigra* və *Helichrysum plicatum* (hər biri 0,15), *Daucus carota*, *Urtica dioica* və *Asparagus officinalis* (hər biri 0,10, ən aşağı İQ) olmaqla sonrakı yerləri tutmuşdur.

Açar sözlər: *Etnobotanika, antiviral bitkilər, immunbanika, immunbərpaedicilər*

Иммуноукрепляющие растения флоры Азербайджана

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Основной целью работы явилось изучение иммуномодулирующих и укрепляющих растений флоры Азербайджана. Частота использования (Ui) и ценность видов растений по их иммуномодулирующим свойствам были оценены новыми методами. Общее количество представленных отдельными лицами данных об использовании каждого конкретного вида обозначено как Ni. Результат соотношения иммуномодуляторов, показан на диаграмме: наиболее высокая IQ (оценка использования) отмечена для *Allium* sp. и *Thymus* sp. (0,90-0,88), следующие места занимают *Rosa* sp. (0,83), *Salvia limbata* (0,81), *Altheae officinalis* (0,80), *Glycyrrhiza glabra* (0,78), *Mathricaria* sp. (0,77), *Humulus lupulus* (0,74), *Mentha* sp. (0,60), *Sitrus* ssp. (0,27), *Capsella burssa pastoris* (0,21), *Rumex* sp. (0,18), *Sambucus nigra* и *Helichrysum plicatum* (каждый по 0,15), *Daucus carota, Urtica dioica* и *Asparagus officinalis* (каждый по 0,10 - самый низкий показатель IQ).

Ключевые слова: *Этноботаника, антивиральные растения, иммуновосстановители*

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A possible method of inhibition of virus COVID-19 reproduction through induction of intra-cellular synthesis and upregulation of interferon I

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The article substantiates studies on the inhibition of virus COVID-19 reproduction within the organism based on upregulation of intracellular interferon I through strengthening synthesis and upregulation of heat shock protein with the molecular mass of 70 kDa.

Keywords: *Virus COVID-19, heat shock protein 70 kDa, intra-cellular interferon upregulation*

Infectious disease, caused by virus COVID-19 and initiated in Wuhan region of China, presently has been spread over more than 200 countries worldwide. Owing to high level of dissemination of virus COVID-19 World Health Organization declared pandemic situation. Naturally, scientists all over the world currently are applying their efforts and modern techniques to elaborate therapy of virus COVID-19.

As it is known from scientific literature, in contrast to the Chinese species, the species of virus COVID-19, widely spread in Europe, are subjected to high level of mutagenicity (Pachetti et al., 2020). On the other hand, significant body of anticipated treatment methods is predominantly related to immunological approaches. In particular, utilization of immunoglobulins to virus COVID-19 provided from the serum of recovalescent patients in therapeutic purposes (passive immunization) or vaccination with virus COVID-19 (active immunization) are based on and utilize antigen-antibody reaction requiring high specificity and mutual recognition between participating agents. Hence, taking into account high level of mutagenicity of European species of virus COVID-19, one can come to a conclusion that in choosing therapy methods of the patients infected with this virus, along with immunological methods application of other methods is important.

Mammalian organisms possess very strong intrinsic protective system and heat shock proteins belong to this system. Being at low levels at normal conditions, under exposure of the organism to adverse factors of different origin immediate (within 3-4 h) launching of synthesis of heat shock proteins is noticed. The results of the studies carried out on different species of animals show that induction of synthesis and upregulation of heat shock proteins having molecular mass of 70 kDa (HSP70) in the tissues is capable of protecting the organism from such severe infectious disease as peritonitis (Allahverdiyeva et al., 2019). At the same time upregulation of HSP70 makes possible to protect somatic cells of the organism from the most part of mutagenic changes induced by damaging impact of high doses of polyaromatic hydrocarbons, poisonous phenol, heavy metals and neonicotinoid insecticides (Mekhtiev and Movsum-zadeh, 2008; Mustafayev and Mekhtiev, 2014; Mekhtiev et al., 2017). HSP70 have enough potency not only in protecting somatic cells from impact of adverse factors in preventive way, but as well in recovery of the already damaged tertiary and secondary structures of denaturized proteins (Ismailova and Mekhtiev, 2018).

As it is known from literature, HSP70 are potent in strengthening intra-cellular synthesis of interferon (Jacquemin et al., 2017). In particular, HSP70 binds specifically to Toll-like receptors 4 (TLR4; Kono and Rock, 2008). TLRs 2 and 4 were originally known as receptors recognizing patho-

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gen-associated molecular patterns, but subsequently it was recognized that TLRs as well are capable of binding to endogenous ligands including HSP molecules (Vabulas et al., 2002). Traumatic brain injury results in high level expression of TLR4 and HSP70 in macrophages/microglia and astrocytes revealed with immunohistochemistry technique. Interestingly, HSP70 was expressed not only in macrophage/microglia and astrocytes but also in neurons, such that injury of any cell type (glia or neurons) could release HSP70 that finally brought to TLR4 signaling (Zhang et al., 2012). Furthermore, ligand-mediated activation of TLR4 also triggers signal transduction for the induction of type I interferon (Kawai and Akira, 2010). Along with the above said, it has been shown in the studies that, in contrast to SARC-1 virus, strengthening of interferon I synthesis bringing to its intracellular upregulation, realizes damaging effect on COVID-19 virus (Vanderheiden et al., 2020; Mantlo et al., 2020; Fig. 1).

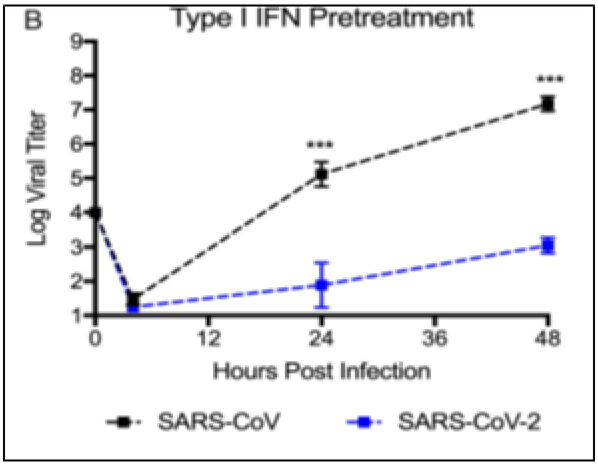


Fig. 1. Sensitivity of COVID-19 virus to type I IFN pretreatment. Vero E6 cells were treated with 1000 units of recombinant type I IFN or mock for 18 hours prior to infection. Cells were subsequently infected at with either SARS-CoV WT (black) or COVID-19 (blue) at an MOI of 1. Each point (n=6) on the line represents the group mean. The two tailed Students t-test: *** - $p<0.001$ (Kumari et al., 2020).

Taking into account the presented data on a whole, one can come to a conclusion that upregulation of HSP70 in the cells of the patients, infected with COVID-19 virus, will make possible realization of effective struggle with it.

In the Academician Abdulla Garayev Institute of Physiology serotonin-modulating anticonsolidation protein was identified first in the rat brain cortex and thereafter purified from the whole brains of the rats and cows (Mekhtiev, 2000). This protein is in linear relation with neurotransmitter serotonin and can realize its functions on sub-cellular level. The studies carried out earlier in our Department on different conditioned models with positive and negative reinforcements, showed that intra-cerebral administration of SMAP to the animals prior to learning sessions brings to significant impairment in memory formation (Mekhtiev, 2000; Guseinov, Mekhtiev, 2013). At the same time the results of our other earlier studies with application of highly sensitive immunochemical Western blotting and ELISA-test techniques showed that within three-hour timeframe from systemic administration of SMAP into the organisms of different animal species, sharp upregulation of HSP70 in their tissues (liver, retina) is observed (Ismailova and Mekhtiev, 2018; Allahverdiyeva et al., 2019).

The studies carried out in our Department demonstrated that upregulation of HSP70 in peripheral tissues can be achieved not only through systemic, but as well through intra-cerebral administration of SMAP to animals. In particular, in the studies conducted over sazan specimens, SMAP administration into the fourth brain ventricle within 4 h brought to significant up-regulation of HSP70 in the liver and back muscles, revealed with ELISA-test ($p<0.001$; Fig. 2).

The said results can be explained in the light of the discovery of peripheral serotonergic nervous system by Prof. V.Smirnov and his colleagues in 2015 y., confirmed by other researchers abroad. According to the results of his pioneer studies, along with sympathetic and parasympathetic nervous systems, peripheral serotonergic nervous system, penetrating all peripheral tissues of the organism, as well exists (Smirnov et al,, 2015). Hence, basing on his data, one can conclude that signal appeared in the brain serotonergic system, via peripheral serotonergic nervous system is delivered to peripheral tissues. At the same time, as other authors have shown, medicine can be delivered directly to the brain (missing blood circulation) through their intra-nasal administration (Pardeshi et al., 2013). After the administration of preparations into the nasal cavity, they reach brain structures within 30-40 min without any loss.

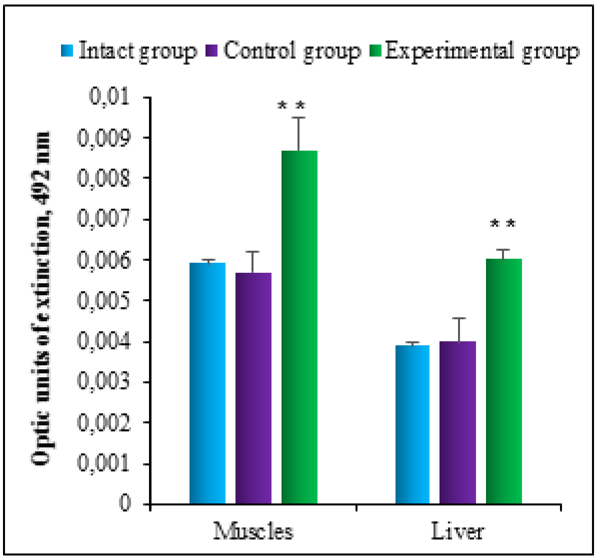


Fig. 2. Effect of administration of SMAP into IV brain ventricle on HSP70 level in the dorsal muscles and liver of sazan specimens. ** - p<0.01.

Concluding the presented data, one can come to an idea that in combatting COVID-19 virus for the purpose of strengthening interferon synthesis and its upregulation inside the cells of the infected patients, it is reasonable and possible to utilize intra-nasal administration of SMAP to the infected patients providing its direct delivery into the brain structures and thereafter upregulation of HSP70 in the peripheral tissues through peripheral serotonergic nervous system.

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İnterferon I-nin hüceyrədaxili sintezinin güclənməsi və miqdarının artırılması vasitəsilə COVID-19 virusunun inhibə etməsinə dair güman olunan üsul

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Məqalədə 70 kDa istilik şoku zülallarının sintezinin gücləndirilməsi və miqdarının artırılması vasitəsilə hüceyrədaxili səviyyədə interferon I miqdarının artırılması əsasında orqanizm daxilində COVID-19 virusunun reproduksiyasının inhibə edilməsi aparılacaq tədqiqatları əsaslandırır.

Açar sözlər: *Virus COVID-19, 70 kDa istilik şoku zülalı, hüceyrədaxili interferon I səviyyəsinin artırılması*

Возможный способ ингибирования репродукции вируса COVID-19 посредством усиления синтеза и увеличения внутриклеточного уровня интерферона I

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В статье приводятся аргументы в пользу проведения исследований по ингибированию репродукции вируса COVID-19 в организме, основанному на повышении внутриклеточного уровня интерферона I посредством усиления синтеза и повышения уровня белков теплового шока с молекулярной массой 70 кДа.

Ключевые слова: *Вирус COVID-19, белки теплового шока с молекулярной массой 70 кДа, повышение уровня внутриклеточного интерферона I*

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Prospective directions for searching new medicines of plant origin, effective in infections of different etiology

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The global community is concerned about the COVID-19 pandemic. Existing capacities are mobilized and new ways to counter the growing threat are actively sought. The scientific development of traditional medicine is a promising way of solving this problem. Information on the use of medicinal plants by different peoples is fragmented and largely unavailable to the world scientific community. The flora of Azerbaijan including almost 1600 species of medicinal plants with antiviral, anti-inflammatory, immunomodulatory, vitamin, general tonic and other properties are distributed in the Flora of Azerbaijan. Modern protocols for the treatment of infection caused by COVID-19, along with other therapeutic agents, will include drugs with the above properties. The article contains information about a computer database of medicinal plants in Azerbaijan, developed by the author in the frame of the doctoral thesis in the 2006 year. These data enable us to distinguish species with a set of biologically active substances that determine their required necessary physiological activity from the total number of medicinal plants. Therefore, the intensification of work on the study of traditional medicine and the creation of a worldwide information platform on medicinal plants can become the basis for the search and development of new antiviral drugs, including those effective and against COVID-19.

Keywords: *Flora of Azerbaijan, medicinal and food plants, database, biologically active substances, physiological activity, COVID-19*

INTRODUCTION

The raging global pandemic caused by the COVID-19 virus has completely changed the way of life of human civilization and showed that the arsenal of modern medicine is not as extensive as we would like to successfully resist this virus. Today, all the forces of the global medical community are mobilized to search for and develop new effective drugs to combat the COVID-19 virus. All possible search directions with at least some prospect of positive conclusion are being developed. Countless scientific organizations are involved in both the synthesis of chemical and the search for natural biologically active substances.

Millennia of traditional healing experience shows that medicines for many diseases are found in nature itself, including in plants that are consumed as food, or used in the form of drugs obtained on their basis. At the same time, modern data indicate that about 20 thousand plant species have been studied for medical purposes, i.e. about 6.5% of all 320 thousand described (Ших и др., 2015). There is no doubt that among the unexplored species there are many plants that can help fight many diseases, including even those that are still considered incurable. The advantage of medicines of natural origin is their gentle effect and relative harmlessness, in comparison with synthetic medicines. In recent decades, synthetic chemistry has become more and more pervasive in all spheres of human life. At present, it is impossible to imagine industry and everyday life without synthetic products - plastics, various dyes, preservatives, flavor enhancers, medical supplies, synthesized drugs, etc.

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Of course, synthetic chemistry has greatly expanded the possibilities of mankind to meet its needs, primarily related to the production of food and medicines, and has improved the quality of life of people. At the same time, it has long become obvious that food products and medicines obtained or processed using the capacities of synthetic chemistry are overwhelmingly inferior to natural products and medicines of natural origin in terms of their environmental purity and human safety.

Therefore, it is not at all surprising that in the modern world the interest of people and the need of medicine for drugs derived from plants and plant materials have greatly increased. However, unfortunately, the modern scientific community does not have wide access to information about plant remedies used in some regions of the world at the local level. And this is mainly due to the fact that today such information is fragmented and still remains out of sight at the global level.

Earlier it was indicated that about 800 species of plants used for medicinal purposes grow in the flora of Azerbaijan (Əliyev, 1998; Дамиров и др., 1988; Dəmirov və b., 1992; etc.). In addition, information about these plants was limited and scattered. Recent studies have established that there are much more such plants and their number reaches almost 1600 species (Ethnobotany of the Caucasus, 2017).

Some chemicals or their natural combinations which are part of medicinal plants are so unique that, despite the achievements of modern science, they cannot be synthesized artificially. Meanwhile, medicinal plants in their natural form and their derived preparations continue to play an important role in health care and their use is part of integrative medicine. Therefore, it is essential to bring this traditional knowledge to the attention of scientists and medical practitioners around the world.

Great opportunities for solving this problem are revealed by modern computer technologies that allow accumulating, storing and ensuring the worldwide availability of such information. In a way, this also solves a certain cultural and historical problem, as it helps prevent the possibility of irrevocable loss of invaluable folk experience.

In the part concerning medicinal plants growing in our country, this problem is partially solved and automated "Electronic Database of Medicinal Plants of Azerbaijan" has been created (Мехтиева, 2006).

This study aims to provide the scientific community with an overview of the research results on the study of the biodiversity of the medicinal flora of Azerbaijan and the prospects for using its capabilities in the search for new therapeutic agents effective for various infections of various etiologies.

MATERIALS AND METHODS

The object of the study was medicinal plants of the flora of Azerbaijan. Analytical information on medicinal plants was obtained through the implementation of the capabilities of the "Electronic Database of Medicinal Plants of Azerbaijan" (Mehdiyeva, 2010). On the basis of the technical assignment prepared by the author, together with the relevant specialists in the Access using DELPHI computer language programming, the Applied Computer program was developed and the electronic database "Medicinal plants of the flora of Azerbaijan" was created. The information array of the Database was formed on the basis of data from almost 500 literary sources on medicinal plants, including monographs, official reference publications, scientific periodicals (peer-reviewed publications), regulatory documents, as well as the results of our own research (Мехтиева, 2015).

RESULTS AND DISCUSSION

As a result of the analysis, it was found that about 1600 plant species with the described medicinal properties, belonging to 742 genera and 179 families, grow in the flora of Azerbaijan. Of these, 274 species are official medicinal plants, 6 species are endemic to Azerbaijan, 77 - endemic to the Caucasus, 44 - relict, and 112 - rare and endangered.

Among the studied medicinal plants of Azerbaijan, many species that contain such biologically active substances (BAS) as flavonoids, alkaloids, vitamin C, essential and fatty oils, anthocyanins, carotenoids, tannins, phenol carboxylic acids, saponins, terpenoids, coumarins, glycosides and lactones have been identified. Moreover, the form and direction of the physiological effects of these and other biologically active substances have been established by experimental and clinical studies.

Such data for some biologically active substances are given in Table 1.

It is the presence of natural biologically active substances in plants that determine their high physiological activity and its direction, including antiviral, antibacterial, antifungal, antioxidant, antihistamine, anti-inflammatory, immunomodu-latory and other effects. Ultimately, only the physiological activity of the substances contained in plants forms their medicinal properties, has a positive effect on metabolic processes, strengthens the immune system, improves the overall state of the organism and mobilizes it to fight the disease (Мехтиева, 2014).

The results of the analysis show that among the medicinal plants of Azerbaijan, there are quite a number of species with medicinal properties in demand in medicine (Fig. 1).

In the context of the considered problem of combating COVID-19, plants exhibiting antiviral, antimicrobial, restorative, anti-inflammatory, immunomodulating and other activity are of particular interest.

Table1. Medicinal plants with predominant groups of biologically active substances and the direction of their physiological activity.

BAS	The number of species with these BAS	Physiological activity, properties	BAS	The number of species with these BAS	Physiological activity, properties
Flavonoids	930	anti-bacterial, antioxidant	Tanning agent	452	bactericide
Alkaloids	597	antifungal	Phenolcarbonic acids	375	antimicrobial, fungistatic, immunomodulating
Vitamin C	518	antioxidant	Saponin	323	anti-bacterial, antivirus, antifungal
Essential oil	468	antimicrobial, antifungal, antivirus	Terpenoids	317	antimicrobial, antifungal, antivirus
Fatty oil	454	anti-bacterial, antivirus, antifungal, immunemodulating	Coumarine	299	antimicrobial, antivirus
Anthocyanin	249	antioxidant, bactericide	Glycoside	139	cardioactive
Carotenoids	220	immunomodulating	Lacton	34	antifungal, antivirus

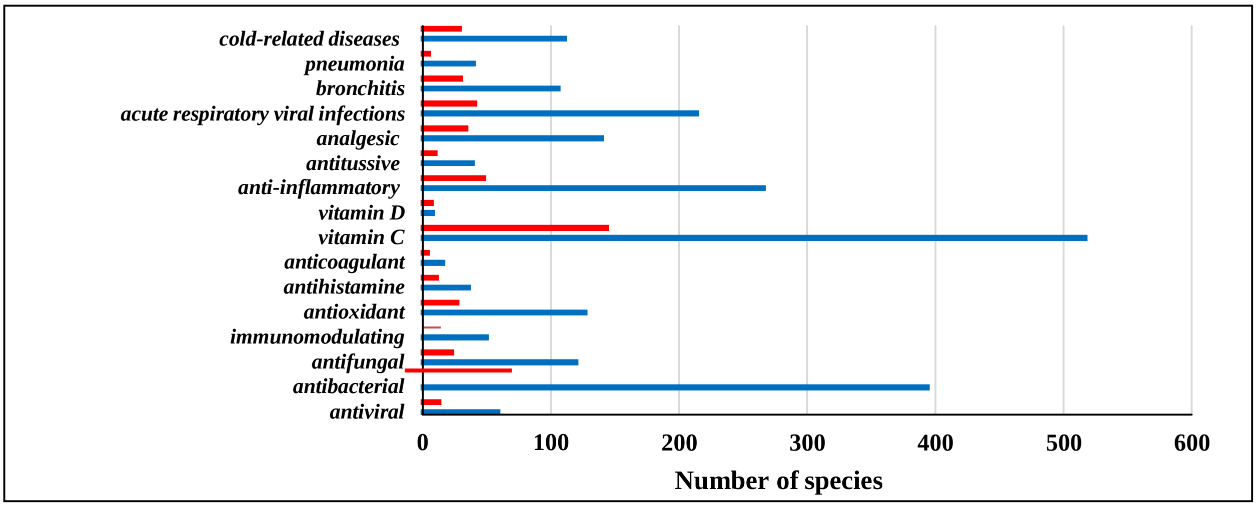


Fig. 1. Quantitative distribution of medicinal and food plants by biological activity and disease.

Table 2. Plants and their derivatives exhibiting physiological activity against certain viral strains.

Virus	Species name	Used parts	Extracts
Herpes types 1 and 2	*Viburnum opulus,* *Verbascum thapsus,* *Melissa officinalis* *Prunella vulgaris*	bark, aerial parts and leaves aerial parts aerial parts	aqueous extract, methanol and alcohol extract aqueous, aqueous-alcoholic extract and essential oil aqueous extract, polysaccharide
Immunodeficiency HIV	*Melissa officinalis*	aerial parts	aqueous extract
Influenza A and B	*Sambucus nigra,* *Verbascum thapsus*	fruit, aerial parts and leaves	aqueous extract, methanol and alcohol
Influenza A2 and B	*Verbascum phlomoides*	flower	decoction
Strains A/PR/8 and A/Hong Kong, FIV, H1N1	*Sambucus nigra*	flower	aqueous extract
Epstein-Barr	*Glechoma hederaceae*		ursolic and olein acid
Ortho- and para-myxoviridae	*Salvia sclarea*	aerial parts	essential oil
Hepatitis C	*Solanum nigrum,* *Lamium album*	seeds aerial parts	methanol and chloroform extract, monoterpene lamiridozin
Tobacco mosaic	*Rubia tinctorum,* *Lonicera captifolium*	aerial part	ethereal extract

Out of the total number of medicinal plants in Azerbaijan, 59 species have been identified for their antivirus activity. It has been established that this activity is manifested mainly in relation to herpes viruses type 1 and 2, HSV-1, HIV immunodeficiency, influenza virus A and B, A2 and B strains, A/PR/8 and A / Hong Kong strains, FIV virus, hepatitis C virus, Epstein-Barr virus, ortho- and paramixoviruses, Koksaki A18 and A21, and tobacco mosaic virus (Liu et al., 2002; Zakay-Rones et al., 2004; Nolkemper et al., 2006; Mazzanti et al., 2008; Roschek et al., 2009; Javed et al., 2011, etc.). Data on the antiviral activity of some of these species are given in Table 2.

Medicinal plants with antibacterial properties is much higher in the flora of Azerbaijan (394 species) (Fig. 1). The extracts and essential oils obtained from them are physiologically active, mainly against bacteria *Echerichia coli*, *Serratia marcescens*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *P. hyogenicum* (Alavi et al., 2006; Mohammad et al., 2007; Цыдендамбаев и др., 2018; Manandhar et al., 2019 etc.).

There are large number of medicinal plants with antifungal properties, numbering over 120 species (Fig. 1). Antifungal activity was determined for extracts and essential oils isolated from these species, mainly against the fungi *Candida albicans*, *Aspergillus niger*, *Mucor circinelloides*, *Fusarium oxysporum*, *Trichoderma lignorum* and *Penicillium funicullosum* (Мустафаева и др., 2006; Sahmurova et al., 2009; Takesh et al., 2019; Giordani et al., 2020 etc.).

Since there are still no effective drugs for direct action against COVID-19, the various treatment protocols for infection caused by this coronavirus mainly consist of methods that stimulate the mobilization of the body's internal resources. For this purpose, drugs with immunomodulatory, anticoagulant, antihistamine, antioxidant and anti-inflammatory effects are used to eliminate the symptomatic manifestations of the disease.

Out of the total number of medicinal plants in Azerbaijan, more than 400 species are at the same time and edible (Мехтиева, 2014; Ethnobotany of the Caucasus, 2017; Alizade et al., 2019). Plants contain a fairly wide variety of biologically active substances with such properties and they enter the body not only in the form of drugs, but also through the direct consumption of plants as food.

For example, out of 59 speciesof medicinal plants with antiviral properties - 13 (*Rhus coriaria*, *Melissa oficinalis*, *Morus alba*, *Juglans regia*, *Mentha aquatica*, *Portulaca oleracea*, *Agrimonia eupatoria*, *Capsicum annuum* etc.); of 394 species with antibacterial properties - 64 (*Allium ursinum*, *Apium graveolens*, *Foeniculum vulgare*, *Eremurus spectabilis*, *Artemisia dracunculus*, *Armoracia rusticana*, *Castanea sativa*, *Ribes nigrum*, *Punica granatum*, *Berberis vulgaris* etc.); of 120 species

with antifungal properties - 23 (*Allium victorialis, Asparagus officinalis, Mentha longifolia, Satureja hortensis, Nigella sativa, Physalis alkekengi, Rumex crispus* etc.) are also food plants.

Food plants, which are also of medicinal importance, are widely used in the national culinary of Azerbaijan. Most of these species are used as spices and seasonings for meat and fish dishes, in marinades, for the preparation of jam, composts, soft drinks, etc., both as fresh and dried. Ultimately, all of these plant species, individually or in combination with other products, contribute, to some extent, to the fight against various diseases and strengthen immunity

Scientists are currently focusing on functional foods and biologically active compounds that support the immune system. A strong immune system is an important factor in the functioning and defense of the body against viral diseases, including those caused by coronaviruses.

To do this, the body needs a sufficient amount of various vitamins and especially C and D. Regular consumption of high-vitamin C and D foods is therefore necessary to maintain immunity.

As the analysis shows, out of 517 species of medicinal plants containing vitamin C - 144 species (*Actinidia deliciosa, Allium sativum, Anethum graveolens, Daucus sativus, Cynara cardunculus, Lactuca sativa, Berberis vulgaris, Armoracia rusticana, Raphanus sativus, Stellaria media, Atriplex hortensis, Cornus mas, Diospyros kaki, Hippophae rhamnoides, Ribes nigrum, Ocimum basilicum, Feijoa sellowiana, Olea europaea, Punica granatum* etc.), and out of 8 species of medicinal plants containing vitamin D - 7 species (*Brassica oleracea, Citrus paradisi, Helianthus annuus, Lycopersicon esculentum, Oryza sativa, Persicaria hydropiper, Spinacia oleracea*) are edible.

At the same time, there are quite a lot of food plants among medicinal plants, which have other medicinal properties. For example, as food products are used:

out of a total of 50 species of medicinal plants with immunomodulatory - 14 species (*Cichorium intybus, Silybum marianum, Salicornia europaea, Panicum miliaceum, Polygonum aviculare, Urtica dioica* etc.);

out of 16 species of medicinal plants with anticoagulant properties - 4 species (*Petasites albus, P. hybridus, Filipendula ulmaria* and *Punica granatum*);

out of 266 species of medicinal plants with anti-inflammatory properties - 48 species (*Allium cepa, Rhus coriaria, Coriandrum sativum, Arctium lappa, Brassica rapa, Salicornia europaea, Spinacia oleracea, Cucurbita maxima, Prunus spinosa, Rubus caesius, Sambucus ebulus, Viola arvensis* etc.**)**;

out of 140 species of medicinal plants with analgesic effect - 34 species (*Pastinaca sativa, Eremurus spectabilis, Crocus sativus, Mentha longifolia, Morus nigra, Nigella sativa, Urtica dioica* etc.);

out of 39 species of medicinal plants with antitussive properties - 10 species (*Cydonia oblonga, Diospyros lotus, Ficus carica, Ocimum basilicum, Morus alba, Thymus transcaucasicus, Hordeum vulgare* etc.);

out of 36 species of medicinal plants with antihistamine properties - 11 species (*Apium graveolens, Berberis vulgaris, Cyperus longus, Lamium album, Mentha piperita, Solanum nigrum* etc.).

Food plants, which are also medicinal, are used for a variety of diseases and their manifestations (Киселева и др., 2007). They are also used as food for:

acute respiratory viral infections - 41 species (*Brassica juncea, Raphanus sativus, Origanum vulgare, Satureja hortensis, Fragaria vesca, Rosa canina, Sorbus aucuparia, Laurus nobilis*, etc.) out of 214 species of medicinal plants;

cold-related diseases - 29 species (*Capsella bursa-pastoris, Capparis herbacea, Beta vulgaris, Crocus speciosus, Rosmarinus officinalis, Rumex confertus, Citrus limon*, etc.) out of 111 species of medicinal plants;

bronchitis - 30 species (*Foeniculum vulgare, Pimpinella saxifraga, Artemisia dracunculus, Helianthus annuus, Fagus orientalis, Thymus kotschyanus, Ziziphus jujube, Citrus unshiu, Vitis vinifera*, etc.) out of 106 species of medicinal plants;

pneumonia - 5 species (*Iris pseudacorus, Rosa canina, Tilia begoniifolia, T. cordata, Viola odorata*) out of 40 species of medicinal plants.

Many of these plants are firmly entrenched in the diet of the population of Azerbaijan and are seasonally, almost daily, used for food, both in natural form and as part of various dishes. According to the available data, some of them are quite effectively

used both as a prophylactic agent for the prevention of diseases and as a drug for the treatment of the disease itself (Franco and Bussmann, 2020). Therefore, it can be assumed that a large plant diversity of the diet of the population of Azerbaijan to a certain extent helps to maintain people's immunity and is one of the elements of the comparatively successful fight against the coronavirus pandemic in our country.

In this context, we believe that one of the main and urgent tasks is the further expansion of research on the study of medicinal plants of the Republic of Azerbaijan. To further saturate the food ration of our people with useful substances, it is necessary to intensify scientific and practical activities for the development and production of new biologically active dietary supplements of plant origin.

CONCLUSIONS

Modern research shows that the spread of certain diseases is often influenced by regional factors, the diet of the population, preferences in tastes and smells, methods and technologies for processing products and, which is very important, but still little studied - genetics and consumer characteristics, for example, the presence of detoxifying enzymes (Rivera et al., 2007; Unnikrishnan et al., 2016; Franco and Bussmann, 2020). Ultimately, understanding the statistics and the nature of the issues associated with these factors will help to better understand how to deal with COVID-19 and other similar viruses.

Summarizing the above, it can be argued that, beyond any doubt, the plant world of Azerbaijan is an inexhaustible source of useful substances, including those that have not yet been identified, and this source probably has a remedy against COVID-19. The pandemic caused by this virus is a global problem and the entire world community is actively seeking means to address it. The key to this question is the basic information platform for the search. However, unfortunately, the vast amount of information about medicinal plants used in some regions of the world at the local level remains largely invisible to scientists worldwide.

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Müxtəlif etiologiyalı infeksiyalara qarşı effektiv olan bitki mənşəli yeni vasitələr axtarışının perspektiv istiqamətləri

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Dünya birliyi COVİD-19 virusunun doğurduğu pandemiya ilə bağlı narahatlıq keçirir. Getdikcə artan təhlükəyə əks təsir göstərmək üçün bütün mövcud imkanlar səfərbər edilmiş və yeni yolların fəal axtarışı gedir. Bu problemin həll edilməsinin perspektivi istiqamətlərindən biri xalq təbabəti təcrübəsinin elmi baxımdan işlənilməsidir. Müxtəlif xalqlar tərəfindən dərmən bitkilərinin istifadəsinə dair məlumatlar pərakəndə xarakter daşıyır və dünyanın elmi birliyi bu bilgilərdən əsasən məlumatlı deyildir. Azərbaycan florasında təqribən1600 növ dərman bitkisi yayılmışdır ki, onların arasında antivirus, antiiltihab, immunmodulləşdirici ümümmöhkəmləndirici, vitaminli və s. bu kimi xüsusiyyətlərə malik bitkilərdə kifayət qədərdir. Digər müalicə vasitələri ilə yanaşı, COVİD-19 virusunun doğurduğu infeksiyanın müasir müalicə protokollarında yuxarıda göstərilən xüsusiyyətlərə malik preparatlar da yer almışdır. Məqalədə, şəxsən müəllifin işləyib hazırladığı Azərbaycanın dərman bitkilərinin komputer bazasında toplanılmış belə bitkilər barədə məlumatlar verilir. Bu məlumatlar bütövlükdə dərman bitkiləri sırasından, zəruri fizioloji fəallığı şərtləndirən bioloji fəal maddələr tutumlu bitki növlərini seçməyə imkan verir. Ona görədə, xalq təbabəti təcrubəsinin tədqiq edilməsi üzrə işlərin fəallaşdırılması və dərman bitkilərinə dair ümumdünya informasiya platformasının yaradılması antivirus xüsusiyyətlərə, o cümlədən COVİD-19 virusuna qarşıda effektiv olan vasitələrinin axtarışı və yeni dərman preparatlarının hazırlanması üçün elmi əsas olabilər.

Açar sözlər: *Azərbaycan florası, dərman və qida bitkiləri, məlumat bazası, bioloji fəal maddələr, fizioloji fəallıq, COVİD-19*

Перспективные направления поиска новых средств растительного происхождения, эффективных при инфекциях различной этиологии

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Мировое сообщество обеспокоено пандемией, вызванной COVID-19. Мобилизованы существующие возможности и идет активный поиск новых путей противодействия продолжающей нарастать угрозе. Перспективным направлением решения этой проблемы является научная разработка опыта народной медицины. Информация об использовании лекарственных растений разными народами носит разрозненный характер и в массе своей недоступна мировому научному сообществу. Во флоре Азербайджана произрастает почти 1600 видов лекарственных растений, в том числе обладающих антивирусными, противовоспалительными, иммуномодулирующими, общеукрепляющими, витаминными и другими свойствами. Современные протоколы лечения инфекции, вызванной COVID-19, наряду с другими лечебными средствами включают и препараты с вышеперечисленными свойствами. В статье приводятся сведения о таких растениях, включенных в компьютерную базу данных лекарственных растений Азербайджана, разработанную лично автором. Эти данные позволяют выделять из общего числа лекарственных растений виды с набором биологически активных веществ, определяющих их требуемую физиологическую активность. Поэтому активизация работы по исследованию опыта народной медицины и создание общемировой информационной платформы по лекарственным растениям может стать основой для поиска и разработки новых антивирусных препаратов, в том числе эффективных и против COVID-19.

Ключевые слова: *Флора Азербайджана, лекарственные и пищевые растения, база данных, биологически активные вещества, физиологическая активность, COVID-19*

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New research directions of bats in Azerbaijan - bats as a potential reservoir of some zoonotic diseases

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Bats - the only flying mammals, accounting for 25% (33 species) of the Azerbaijani mammalian fauna. Those have also been focused on by the scientific community, zoologists, veterinarians, and virologists for the recent 20 years because of their assumed or confirmed role in the spillover of several new zoonotic diseases. Such studies have not been carried out so far in Azerbaijan and the situation with the study of other bats diseases is not satisfactory. Thus, the national studies on bats on ecto-, endo- and blood-parasites require at least an update, dating back to the 70s of the last century. This paper provides an overview of the potential or confirmed reservoir role of bats in a number of pathogens, the importance of research in this area and, in particular, the first research attempts in coronary studies conducted in Azerbaijan within the frame of Western Asia Bat Research Network.

Keywords: *Bats, zoonotic diseases, parasites, sampling site, colonies, Western Asia*

INTRODUCTION

Bats have attracted the attention of the scientific community over the last 20 years due to the possibility of being a natural reservoir of some viruses (especially coronaviruses) and have become the subject of relevant multi-disciplinary research projects (Kuzmin et al., 2011). The epidemiological side of bats, especially probability of them as potential natural reservoir of viral diseases has never been studied in Azerbaijan. In addition to general faunal and zoological researches, the study of bats from different ecological groups and species as a natural reservoir in the spread or spill over of ecto, endo- parasites, as well as viral diseases is of great importance. As part of a regional multidisciplinary study in Western Asia, investigations being conducted in Azerbaijan will determine the role of local bat fauna representatives on the regional epidemiological map of bat borne diseases.

MATERIALS AND METHODS

As a first step, bats were obtained by dedicated mist (japan) nets at the entrances of shelters (anthropogenic and natural habitats) during evening fly out and morning return into the roosts via application of standard techniques (Kunz et al., 1996). In some cases, individuals were captured from summer shelters manually or by using special catchers-butterfly nets. Each individual caught was first identified up to the species level, age and sex of animal were determined by generally accepted methods.

A blood sample (from one of the large vessels of tail), an oral swab, and rectal swab (in absence of excrement) were then taken from each individual and fixed in an RNA-Later solution (Fig. 1). Smaller wing punches were also taken for further DNA analysis for species having controversial taxonomy. All samples were stored at -80° C (in cruioshipper with nitrogen solution) and then transferred into an ultra-cold refrigerator for further storage until lab analysis execution.

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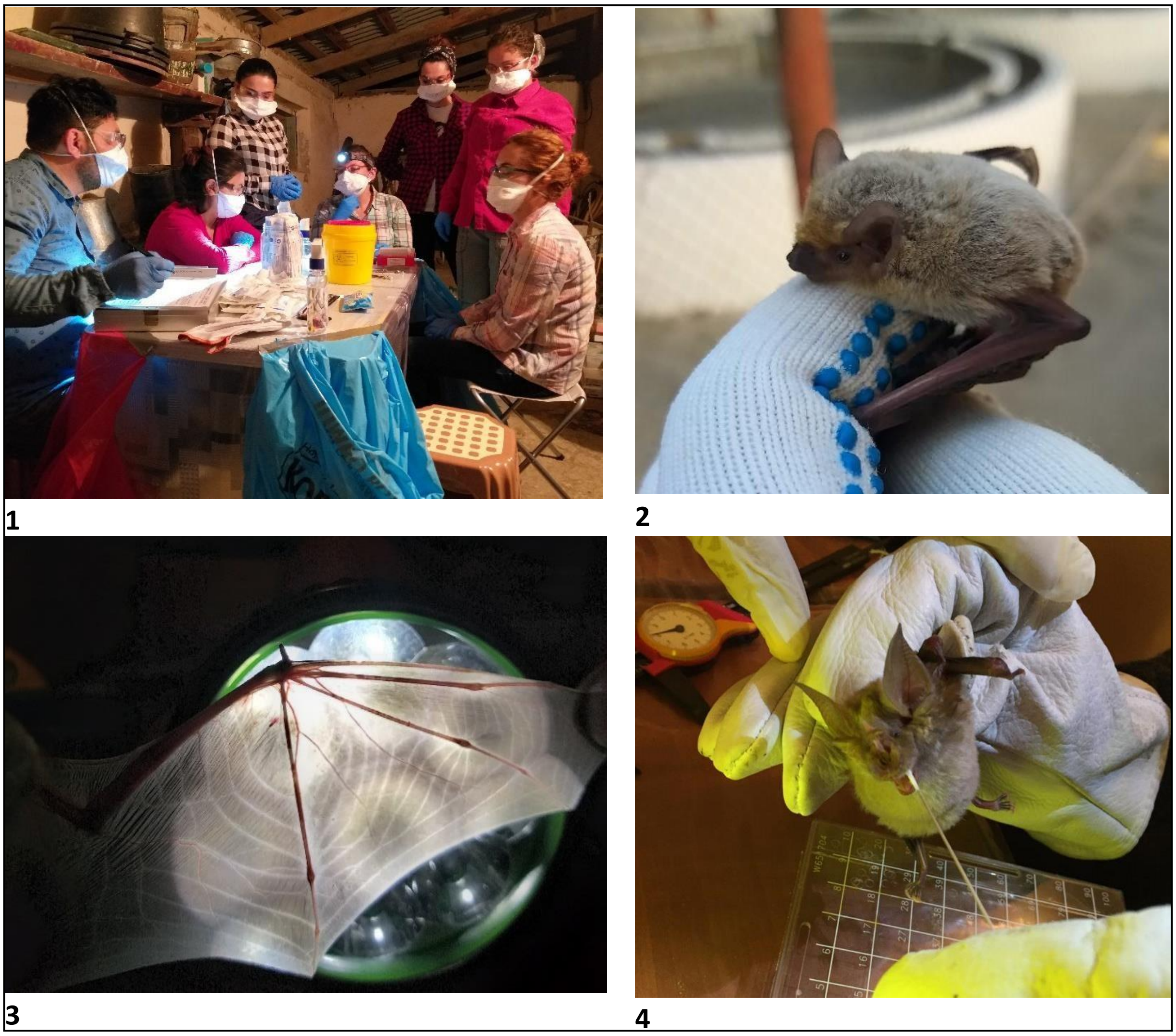


Fig. 1. Sampling: (1– team, 2 – *Pipistrellus kuhlii*, 3 – age determination via wing bones, 4 – oral swab taking from *Rhinolophus ferrumequinum*).

To determine ectoparasites of bats, beetles and other insects from the fur of individuals were collected and put into special solutions, blood films (peripheral blood smear) were prepared from collected blood samples and fixed in 70% methanol, excrements were fixed in alcohol solution (Dryden et al., 2005; Houwen, 2020).

Blood parasites and ectoparasites will be identified by the specialists of the Institute of Zoology, viral (coronoviruses) analysis will be carried out in the Georgian NCDC laboratory by application of appropriate methods. According to the protocol, 90 individuals were obtained from each sampling site (bat colony), and upon completion of sampling the bats were released.

Three sampling sites: 1. Greater Caucasus (Gakh city), 2. Lankaran Natural Province (Fishermen's settlement near the Kyzylagach reserve) and 3. Caspian lowland (Neftchala district, Mayak settlement) were visited over the period of 2019-2020 for bats' samples collection (Fig. 2).

RESULTS AND DISCUSSION

Among the diseases of bats in Azerbaijan, their parasitic fauna has been studied the most. In 1966, research on 700 individual bats of 15 species revealed 24 helminth species (Shakhtakhtinskaya et

al., 1971; Sadikhov, 1978). The most common helminth-infected species were *Rhinolophus ferrumequinum* (greater horseshoe bat) and *Nyctalus noctule* (noctule bat). Blood parasites were found in some local bat species (Zeyniyev, Rakhmatulina 1990) and infection was recorded in 70.3% of 30 individuals belonging to eight species. Four types of blood parasites: *Trypanasoma*, *Babesia*, *Grahamella*, *Micrafilaria* were registered and it was confirmed that the frequency of invasion was low in infected individuals, and in some cases, individual parasites were found.

Weak coccidial infections in bats have also been reported (Musayev, 1976; Musayev, Gauzer 1971). *Eimeria vespertilii* Musayev and Veysov, 1961; *E.zakirica* Musayev, 1967, and *E. mehelyii* sp.n species are described in *Rhinolophus mehelii*.

Ectoparasites have been better studied in bats (Dubovchenko, 1969; Mulyarskaya, 1978; Hadjiyev et al., 1982). From 1,572 individuals of 11 species, 97.3% were found to be infected with 90 species of ectoparasites, of which 91% were ticks, 36.2% were blood-sucking flies, 19.1% were fleas, and 1.7% were lice. A richer ectoparasite fauna has been revealed among the more densely colonized bats. The majority of ectoparasites (97.2%) were specific to bats only and were not similar to the ectoparasitic fauna of birds and rodents. According to Dubovshenko (1976), more than 20 of the 90 ectoparasite species in bats of Azerbaijan might be carriers or reservoirs of infectious and invasive pathogens.

Unfortunately, the potential role of bats in the spread of viral diseases in Azerbaijan has not been assessed. Although rabies has been recorded in bats for more than a century, the carrier of some pathogens that have emerged in recent years (lissaviruses, henipaviruses, coronaviruses) have been confirmed to be bats, while others (filoviruses) are still being assumed to be bats. New pathogens are being recorded in bats.

Rabies is caused by lissaviruses of the *Rhabdoviridae* family and is characterized by the highest mortality rate. For 10 of the 11 species of lissavirus, bats play a major reservoir role (Kuzmin et al., 2011). While the classical rabies virus (RABV) circulates only in American bats, more different types of lissavirus (EBLV-2 and EBLV-2) are known in Europe from 3 bat species (*Eptesicus serotinus, Myotis daubentonii, M. dasycneme*), two of which are widespread in our country. *E. serotinus* is a common species and is closely related to manmade structures, as well as forming mixed colonies with *P. kuhlii*, the most common type of synanthropic bat.

Filoviruses are known as Marburg (MARV) and Ebola (EBOV) viruses. The geography of the first disease is assosiated with Africa and Sahara, and although the virus was recorded only in fruit-eating bats in 2001-2005, the MARV virus was later reported in Congo, in bats of *Miniopterus* and *Rhinolophus* genuses. Representatives of both genused are included into bat auna of Azerbaijan.

Severe Acute Respiratory Syndrome (SARS), which has been known to cause coronaviruses (CoV) since 2002, has also been reported in bats (and other mammals). Later, SARS-like viruses were found in bats in Europe, South America, Australia and Africa. Alpha and beta coronoviruses, known from bats of the Vespertilionoidae family, have been reported to be genetically similar to the coronovirus described from China. The basis of the fauna of Azerbaijani bats are the species of the family Vespertilionoidae (25 out of 33 species).

The geographical position of Azerbaijan has led to the diversity of bats, and has created a basis for the combination of different bat species with different zoogeographical origin (Fig. 3). From 33 local bat species, 41% are Trans-Paleoarctic, 35% East Asian arid and temperate, 15% European, and 9% Turan desert and semi-desert species (Rakhmatulina, 2005). Studying of bats and assosiated diseases (in migrant and sedentary species) is even more important in such an environment. Particularly, it is important to take preventive measures against viral diseases that may be dangerous to human health.

Bearing in mind the above mentioned note, the first field research on the study of coronoviruses in bats was launched in Azerbaijan in 2019 within the West Asian Bat Network (WAB-Net) project, and the second part of the research was completed in August and September of 2020. It is planned to inspect bats from 3 more new sampling sites during summer-autumn season of 2021. In accordance with protocol requirements, 90 individual bats were obtained from each sampling site, relevant rectal, oral swabs, excrement, blood were collected as well as wing punches (DNA samples) were taken after which the animals were finally released.

In September 2019, two species (*P. kuhlii* - 88 individuals and *P. nathuisii* - 2 ind.) from a sampling site in Mayak settlement of Neftchala region, in August 2010, 4 species (*P. kuhlii* - 29 ind., *P. pipistrellus* - 40 ind., *P. nathuisii* - 20 individuals and *Eptesicus serotinus* - 1 individual) from a sampling site at Balikchylar settlement of Lankaran region, and in September 2020, 4 more species (*P. pipistrellus* - 1 ind., *Myotis emarginatus* - 47 ind., *Rhinolophus ferrumequinum* - 37 ind. and *Rh. hipposideros* - 5 ind.) from sampling site in Gakh town were inspected (Table 1).

The target of the field works is bats from different ecological groups, both for synanthropic species associated with manmade structures and natural shelters (caves, rocks, tree hollow etc.). The research will be continueing in the coming years and will cover bat species from different natural provinces of the country. As similar studies will be conducted in the South Caucasusian and a number of Western Asian countries by the end of 2021, the role of Azerbaijani bats in the epidemiological situation of coronaviruses in the region will be clarified. Laboratory tests will continue until the end of 2021, and the results will be published and posted.

In order to look for ecto- and endo (blood)-parasites of bats, blood films (peripheral blood smear) were prepared from 50 bat individuals blood samples, including the species *Myotis alcatoe* (2 individuals) and *Pipistrellus kuhlii* (8 individuals) captured in Sitalchay settlement of Khizi region and Chayli village of Gobustan in addition to 7 species (270 individuals) of bats obtained for viral investigations. 200 smears were prepared on the basis of individual blood samples, ectoparasites from 30 individual bats were isolated and collected for further identification.

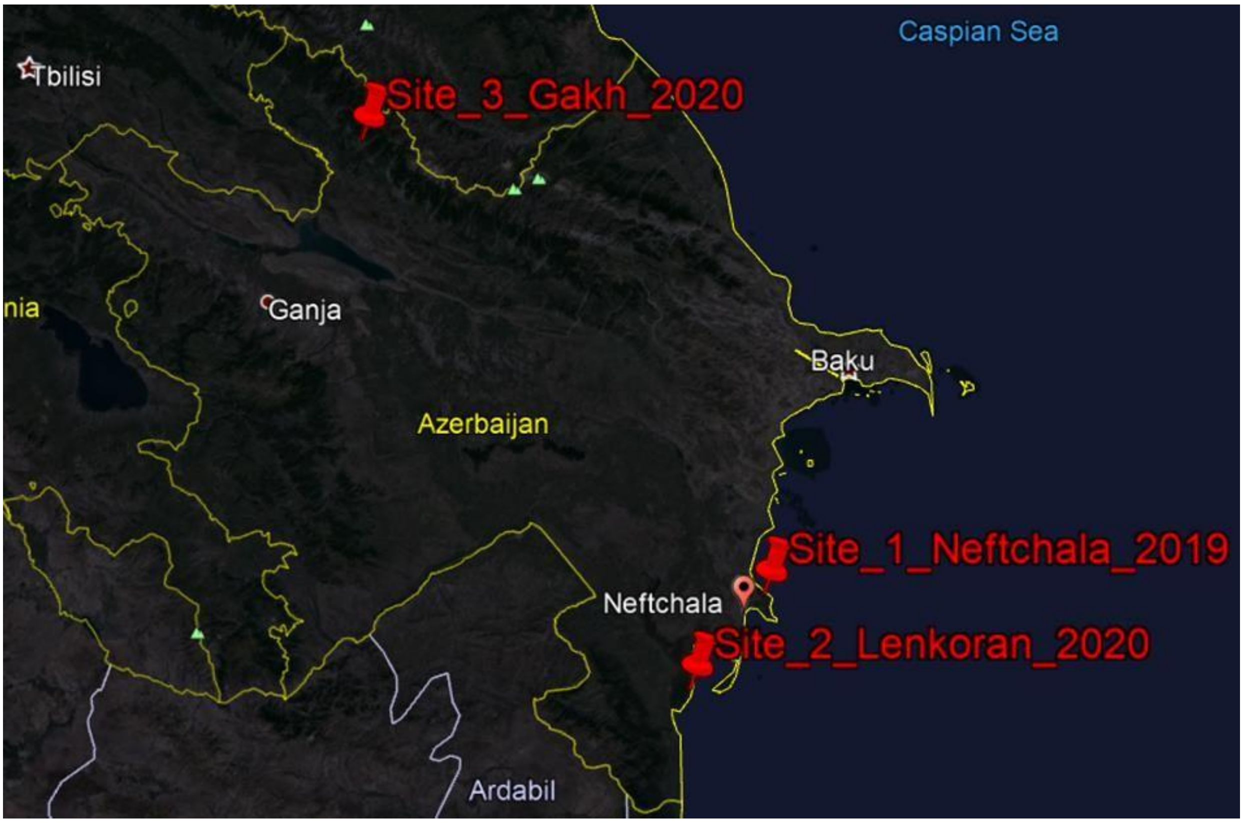


Fig. 2. Sampling sites of bats in Azerbaijan (period of 2019-2020).

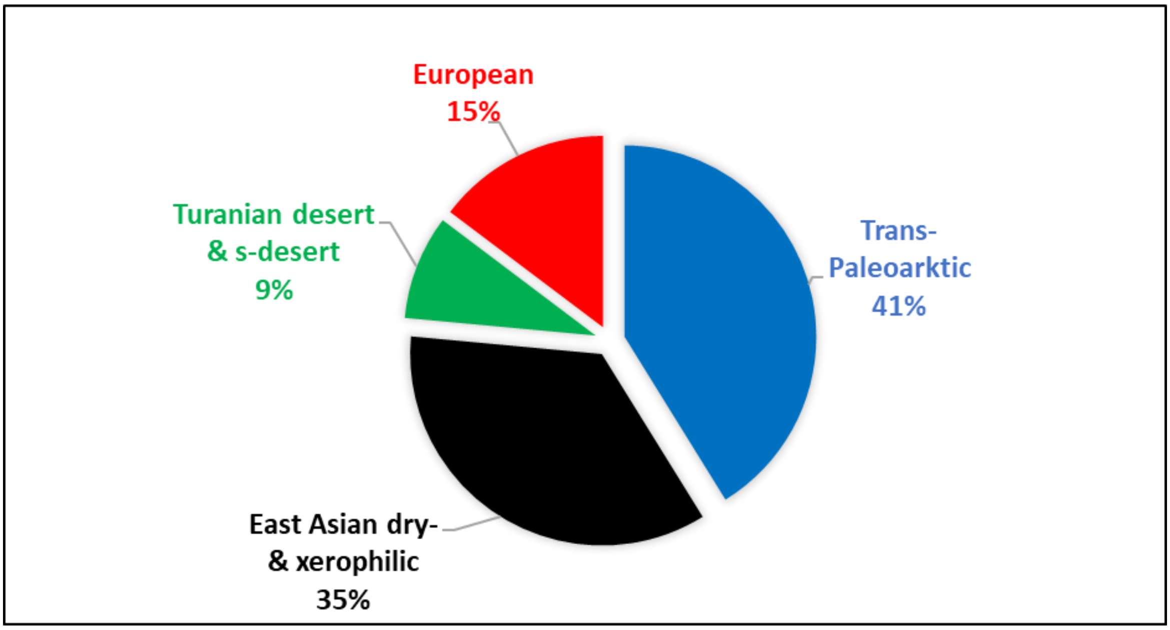


Fig. 3. Zoogeographical origin of bats in Azerbaijan.

Tab.1. Bats species and number of individuals captured fc r further analysis o\ er the period of 2019-2020.

Bat species			Site 1: Neftchala	Site 2: Lenkoran	Site 3: Gakh
	Scientific name	Common name	Number of individuals		
1.	*Pipistrellus kuhlii*	*Kuhl's pipistrelle*	88	29	0
2.	*P. pipistrellus*	*Common pipistrelle*	0	40	1
3.	*P. nathusii*	*Nathusi'spipistrelle*	2	20	0
4.	*Eptesicus serotinus*	*Serotine bat*	0	1	0
5.	*Myotis emarginatus*	*Geoffroy's bat*	0	0	47
6.	*Rhinolophus ferrumequinum*	*Greater horseshoe bat*	0	0	37
7.	*Rh. hipposideros*	*Lesser horseshoe bat*	0	0	5

These studies aslo revealed new summer and winter roosts of greater and lesser horshore bats (*Rh.ferrumequinum* and *Rh.hipposideros*), as well as Geoffroy's bat (*Myotis emarginatus*) in the Greater Caucasus, summer colony of alcato bat (*Myotis alcatoe*) in Khyzi and Gobustan region. These are the new addendums onto the distribution maps of bats in Azerbaijan

CONCLUSIONS

1. The first- and second-year bat sampling studies for inspection of bats as potential natural reservoir of coronaviruses have been completed in Azerbaijan.
2. In total, 270 individuals from seven species of bats were obtained from three natural geographic provinces. Blood samples, oral and rectal swabs (or excrement), and wing punches (DNA samples) were obtained from each individual for the cases if extra taxonomic identification will be required, respectively.
3. In parallel, samples for ecto-, endo-, blood parasites of bats captured have been collected for further cameral analysis, which will refresh and fulfil the aged (50-60 years old) data and much more local bat species will be inspected. Local bat species were last studied in the 1960s. Ecto-parasites of only

11 species, blood parasites of eight bat species, helminths of 15 species of bats were investigated.

4. For the first time, new winter and summer roosts of four bat species (greater and lesser horseshoe bats, Geoffroy's bat and Alcato bat) were revealed in the Greater Caucasus.

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Azərbaycanda yarasaların yeni tədqiqat istiqamətləri - yarasalar bir sıra zoonoz xəstəliklərin potensial rezervuari kimi

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Azərbaycan məməlilər faunasının 25%-i (33 növ) yeganə uçan məməlilər olan yarasalar sinfinin payına düşür. Yarasalar həm də bir sıra yeni aşkar olunan zoonoz (virus) xəstəlikərin yayılmasındakı roluna görə xüsuslıə son 20 ildə elmi ictimaiyyətin, zooloq, veterinar, virusuloqların diqqət mərkəzində olub müvafiq kompleks tədqiqat layihələrinin obyektlərinə çevirlimişlər. Bu kimi tədqiqat işləri ölkəmizdə həyata keçirliməyib və yarasaların digər xəstəliklərinin öyrənilməsi vəziyyəti də qənaətbəxş deyildir. Beləki, yarasaların ekto-, endo- və qan parazitləri üzrə respublikada aparılmış araşdırmaları keçən əsrin 70-ci

illərinə təsadüf etdiyindən yenilənmə tələb edir. Məqalədə yarasaların bir sıra virus xəstəliklərinin potensial və ya təsbit olunmuş rezervuar rolunun əhəmiyyəti, bu sahədə araşdırmaların vacibliyi və xüsusilə korona-virusların öyrənilməsi sahəsində həyata keçirilən ilkin işlər haqqında məlumat verilir.

Açar sözlər: *Yarasalar, zoonoz xəstəlikləri, parazitlər, nümunəgötürmə sahələri, koloniyalar, Qərbi Asiya*

Новое направление исследований летучих мышей в Азербайджане - летучие мыши как потенциальный резервуар ряда зоонозных заболеваний

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Летучие мыши, являющиеся единственными летающими млекопитающими, составляют 25% фауны млекопитающих Азербайджана (33 вида). В течение последних 20 лет летучие мыши были в центре внимания научного сообщества: зоологов, ветеринаров и вирусологов, особенно из-за их роли в распространении ряда недавно открытых зоонозных (вирусных) болезней. Подобные исследования в нашей стране не проводились, и ситуация с изучением других болезней летучих мышей остается неудовлетворительной. Таким образом, исследования экто-, эндо- и кровяных паразитов летучих мышей в стране требуют обновления, так как относятся к 70-м годам прошлого века. В статье представлена информация о потенциальной или установленной резервуарной роли летучих мышей в ряде вирусных заболеваний, важности исследований в этой области и, в частности, о начальной работе, проводимой для изучения коронавирусов.

Ключевые слова: *Летучие мыши, зоонозы, паразиты, пункты отбора проб, колонии, Западная Азия*

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Effect of vitamin D blood levels on the disease in patients with COVID-19

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The danger of COVID-19 infection is its higher contagiousness (several times higher than influenza), a long incubation period (up to 14 days), and is complicated by the fact that patients without any symptoms are carriers of the infection. Currently, the clinical and epidemiological researches on the characteristics of the disease continue, along with the development of the new means of its prevention and treatment. The most important preventive resource is the activation of the antiviral innate immune system. According to the literature, an adequate supply of vitamin D is one of the foundations of the antiviral immune system, including against the influenza virus. No clinical trials have been conducted to determine the relationship between vitamin D blood level and clinical outcome in COVID-19 patients.

To study the effect of blood levels of vitamin D in patients infected with COVID-19 on the course of the disease and the outcome of treatment, a medical history of 19 patients with laboratory-confirmed COVID-19 infection was analyzed. The patients were treated at the «MediClub» LLC between 15.06.2020 - 15.07.2020. «MediClub» hospital was approved by «TABIB» Azerbaijan for the treatment of patients with COVID-19. Data on clinical manifestations and blood 25-hydroxyvitamin D [25(OH)D] levels were obtained from all medical histories.

A retrospective analysis of laboratory-confirmed cases of COVID-19 showed a relationship between the blood level of 25-hydroxyvitamin D [25(OH)D] and the nature of the course of COVID-19 and the development of complications of the disease.

Taking into account the main cause of critical complications of COVID-19 is impaired immunity, and cholecalciferol harmonizes the functioning of the immune system at all its levels, it can be concluded that an adequate level of vitamin D in the blood increases the likelihood of a light course of COVID-19 and its favorable outcome.

Keywords: *Coronavirus infection, pandemic, COVID-19, 25-hydroxyvitamin D [25(OH)D], blood level of vitamin D, volume of lung damage, proinflammatory cytokines, concomitant diseases*

INTRODUCTION

The coronavirus infection COVID-19 was officially registered by the World Health Organization (WHO) on December 31, 2019, when the PRC Ministry of Health reported 44 cases of SARS in Wuhan City, Hubei Province. On February 11, 2020, the WHO determined the official name of the infection caused by the new coronavirus - COVID-19 ("Coronavirus disease 2019"). On February 11, 2020, the International Committee on Taxonomy of Viruses stated the official name of the infectious agent, a SARS-CoV-2. It has been established that COVID-19 is caused by the new coronavirus SARS-CoV-2. Later, on March 11, 2020, WHO declared a COVID-19, a pandemic (Громова и др., 2020; Временные методические рекомендации: профилактика, диагностика и лечение новой коронавирусной инфекции (COVID-19), 2020).

The danger of COVID-19 infection is its higher contagiousness (several times higher than influenza), a long incubation period (up to 14 days)

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and is complicated by the fact that patients without any symptoms are carriers of the infection. COVID-19 is characterized by a severe course in the presence of the chronic pathology in patients. These COVID-19 features place increased demand on the healthcare system. In particular, the higher contagiousness leads to the simultaneous disease incidence of COVID-19 of the large number of people, which has led to the overload of the health care system in several countries. More severe course of infection in patients with a chronic pathology is associated with use for adaptive lung ventilation (ALV) and with a higher mortality rate (Громова и др., 2020).

Currently, the clinical and epidemiological researches on characteristics of the disease continues, along with the development of the new means of its prevention and treatment. The most common clinical manifestation of the COVID-19 is bilateral pneumonia (diffuse alveolar damage and microangiopathy); acute respiratory distress syndrome (ARDS) was recorded in 3-4% of the patients. Some patients develop hypercoagulable syndrome with thrombosis and thromboembolism, along with the other damage of the organs and systems (the central nervous system, myocardium, kidneys, liver, gastrointestinal tract, endocrine and immune systems), and also sepsis and septic shock may be developed.

Attempts to control the COVID-19 only by quarantine measures (for example, wearing masks, gloves, washing hands, social distancing, self-isolation, and other) do not engage the most important preventive resource such as the activation of antiviral innate immune system. This aspect is especially important in the case of COVID-19, as this infection is highly contagious and can lead to severe pneumonia and acute respiratory failure. To identify risk groups for a severe course of the disease, it is necessary to systematize the features of the pathogenesis of COVID-19, which distinguish it from other coronavirus infections (Alipio, 2020).

Vitamin D is one of the most important immunity regulators. Adequate supply of vitamin D is one of the foundations of anti-viral immune system, including against the influenza virus. It has been proven that vitamin D reduces the risk of acute respiratory viral infection (ARVI) (Rondanelli et al., 2018). Vitamin D deficiency is associated with impaired functioning of innate and adaptive immune systems, also with increased risk of viral and bacterial diseases. A patient of any age with vitamin D deficiency develops chronic inflammation, which significantly reduces the body's resistance to bacterial and viral diseases (ARVI, influenza, rhinitis, bronchitis, obstructive pulmonary diseases) (Громова и др., 2020). In addition, vitamin D increases cell-mediated immunity (Cantorna, 2010), modulates adaptive immune system (Sharifi et al., 2019) and increases the expression of genes encoding the antioxidant enzymes (Lei et al., 2017). Therefore, several authors have proposed the use of vitamin D for the prevention and treatment of COVID-19 (Alipio, 2020). No clinical trials have been conducted to determine the ability of vitamin D to suppress the COVID-19 virus. There is a lack of tests for the statistically significant relationship between vitamin D levels and clinical outcome in COVID-19 patients. In this article, we have used vitamin D status to predict clinical outcomes in patients infected with COVID-19. The analysis was based on the level of 25-hydroxyvitamin D [25(OH)D], a measure of the amount of vitamin D in the body.

Purpose of the study: To study the effect of blood levels of vitamin D in patients infected with COVID-19 on the course of the disease and the outcome of treatment.

MATERIALS AND METHODS

We analyzed medical history of 19 patients with laboratory confirmed COVID-19 infection. The patients were treated at the «MediClub» LLC between 15.06.2020 - 15.07.2020. Hospital «MediClub» was approved by «TABIB» Azerbaijan for the treatment of patients COVİD-19. Data on clinical manifestations and blood 25-hydroxyvitamin D [25(OH)D] levels were obtained from all medical histories. It should be noted that the patients we analyzed were inpatients. They were hospitalized due to the severity of the physical condition. Thus, all the patients had respiratory failure and increased body temperature. The clinical analyzes were obtained from the patients, presentation of pulmonary infection on computer tomography (CT). It was important to obtain the level of 25-hydroxyvitamin D [25(OH)D in the blood of the patients and to assess their clinical condition in accordance with the obtained data.

RESULTS AND DISCUSSION

A total of 19 patients with COVID-19 enrolled to this study, where 11 were men and 8 were women (Fig. 1). Most of the patients were men (58%).

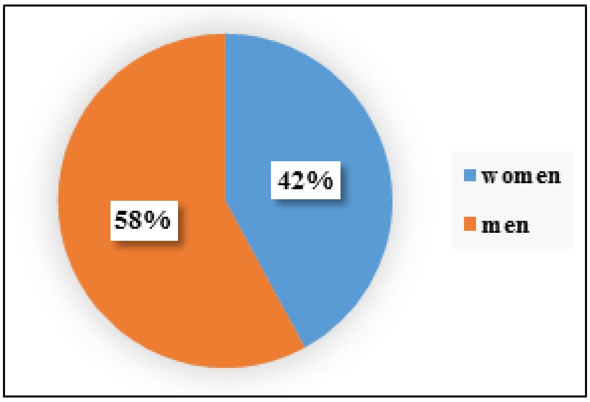


Fig. 1. The total number of patients participating in the study (total 19 patients).

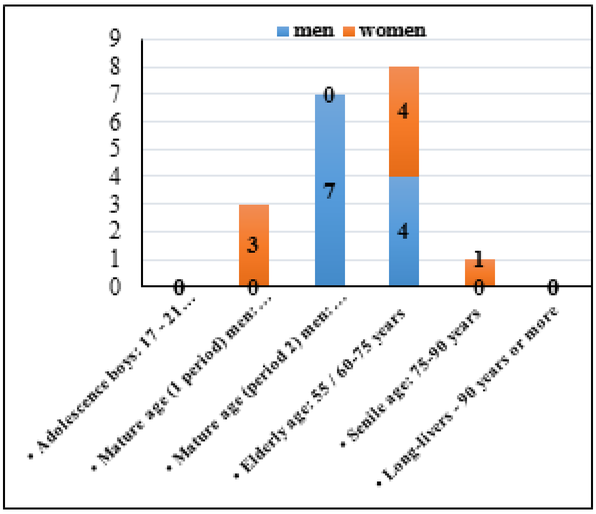


Fig. 2. Distribution of patients participating in the study by age (total 19 patients).

The age distribution of the patients was following (Fig. 2):

- adolescence boys: 17-21 years, girls: 16-20 years;
- mature age (1 period) men: 21-35 years, women: 20-35 years;
- mature age (period 2) men: 35-60 years, women: 35-55 years;
- advanced age: 55/60-75 years;
- senior adult: 75-90 years;
- long-livers - 90 years or more.

In our study, the overwhelming number of patients (15 patients) belongs to mature age (period 2), 37% and to old age, 43%, which amounted to 80% in general.

The patients' distribution by the volume of lung damage is presented in the Fig. 3.

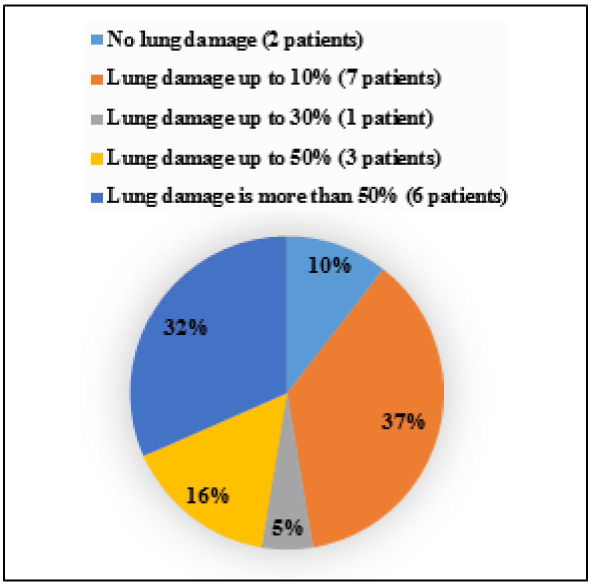


Fig. 3. Distribution of patients participating in the study by the volume of lung damage (19 patients in total).

According to the volume of lung damage, confirmed by CT of the chest, the cases were classified as follows:

- COVID-19 infected patients with clinical manifestations, without lung involvement;
- COVID-19 infected patients with clinical manifestations, the volume of lung damage was up to 10%;
- COVID-19 infected patients with clinical manifestations, the volume of lung damage was up to 30%;
- COVID-19 infected patients with clinical manifestations, the volume of lung damage was up to 50%;
- COVID-19 infected patients with clinical manifestations, the volume of lung damage was more than 50%.

Patients with severe clinical manifestations (hypoxia (oxygen saturation below 93%), with respiratory impairment or deviations in the results of laboratory blood gas analyzes (PaCO2> 50 mm Hg Art.), and respiratory failure requiring intensive monitoring of the patient) accounted for 48% (9 patients) of the total number of the patients. These were patients whose volume of lung damage was up to 50% (3 patients - 16% of the total number of patients) and patients whose lung damage was more than 50% (6 patients - 32% of the total number of patients).

An important criterion, in the presence of which the health status of COVID-19 patients is aggravated, is the presence of concomitant diseases. It is known that the presence of any chronic inflammation in a patient (glomerulonephritis, cholestasis, atherosclerosis, obesity, diabetes mellitus, bronchial asthma, endothelial dysfunction in hypertension, and other) stimulates more rapid increase in the synthesis of proinflammatory cytokines. We analyzed the relationship between the presence of concomitant diseases with the spread of the pathological process in the lungs (Fig. 4).

Eight patients (42%) had concomitant diseases, which is a serious complication associated with COVID-19. Six patients with severe lung damage (lung damage is more than 50%) and, thus, with a severe clinical course of COVID-19, suffered for many years from various somatic diseases, such as diabetes mellitus, atherosclerosis, coronary heart disease, coronary angioplasty, arterial hypertension, chronic renal failure, obesity. All patients had severe respiratory failure, 4 out of 6 patients had acute respiratory distress syndrome (RDS). All patients at certain stages of treatment were connected to the AVL. Two of these patients with volume of lung damage up to 95% and 100% died.

The blood levels of vitamin D in patients with COVID-19 in the study has been classified based on serum 25 (OH) D levels:

- <10 ng/ml (severe vitamin D deficiency);
- 10-20 ng / ml (vitamin D deficiency);
- 20-30 ng / ml (vitamin D insufficiency);
- 30-100 ng / ml (adequate level of vitamin D);
- 100 ng / ml (excess vitamin D).

The obtained data of blood levels of vitamin D in patients is presented in the Fig. 5.

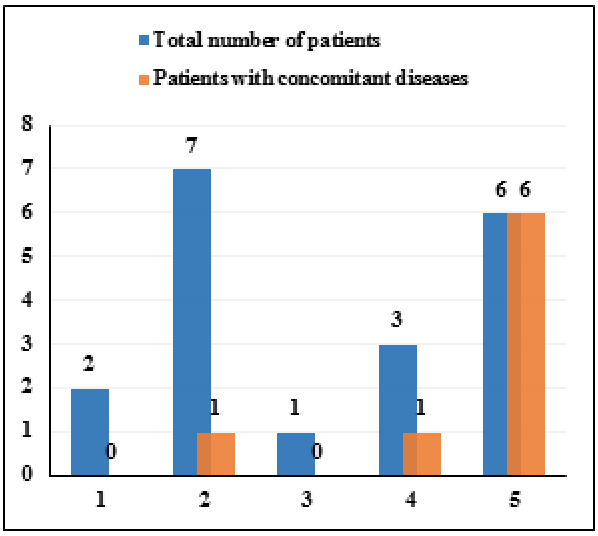


Fig. 4. Patients with the presence of concomitant diseases, respectively, the spread of the pathological process in the lungs (19 patients in total).

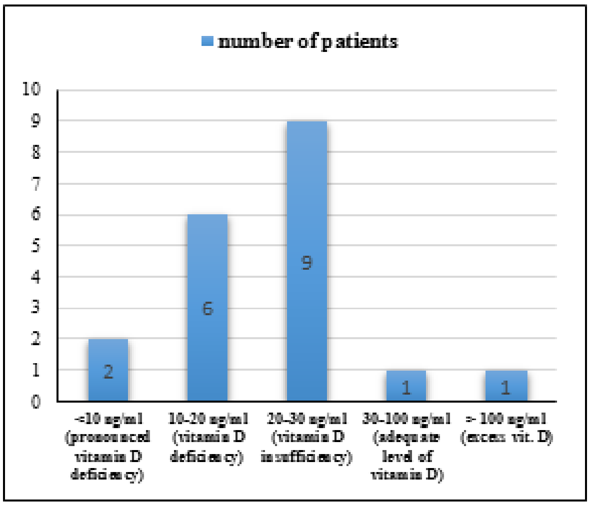


Fig. 5. Vitamin D status in the blood of the studied patients (total 19 patients).

The analysis showed that 15 (79%) of the total number of patients had a deficiency (6 patients - 32%) and insufficiency (9 patients - 47%) of vitamin D in the blood. Two patients (11%) had a (pronounced vitamin D deficiency (<10 ng/ml).

The relationship between the blood levels of vitamin D with the volume of lung damage is presented in Table 1.

Table 1. Vitamin D status in the blood of the studied patients, depending on the degree of lung damage (19 patients in total)

	<10 ng/ml	10-20 ng/ml	20-30 ng/ml	30-100 ng/ml	> 100 ng/ml
No lung damage (2 patients)	0	0	2	0	0
Lung damage up to 10% (7 patients)	0	4	2	0	1
Lung damage up to 30% (1 patient)	0	0	1	0	0
Lung damage up to 50% (3 patients)	0	0	2	1	0
Lung damage is more than 50% (6 patients)	2	2	2	0	0

Table 2. Statistical analysis results.

Number of observatrions	17
degrees of freedom	15
correlation coefficient	-0,581
t-statistic	2,765
p-value	0,014

We employ statistical analysis to examine the relation between the level of vitamin D and the lung damage of the 19 observed patients. Due to possible distortions, we exclude the 2 outliers with the vitamin D level of 100 and 38.6, because they used drugs containing vitamin D for prophylactic purposes long before they became infected with COVID-19. This leaves us with the population of 17 observations.

As both variables are continuous, we utilize the Pearson correlation test. We obtain the Pearson correlation coefficient of -0.581 (negative correlation). The t-statistic of the corresponding two-tailed test is 2.765, with the respective p-value of 0.014. As the p-value is substantially less than 0.05, we can conclude that the correlation is significant (Table2).

One patient had blood levels of vitamin D more than 38.2 ng / ml and only one patient had the vitamin D level > 100 ng/ml. It was noted that patients used drugs containing vitamin D for prophylactic purposes long before they became infected with COVID-19. The remaining 17 patients (89,5 % of total) had blood levels of vitamin D below 30 ng/ml (vitamin D deficiently).

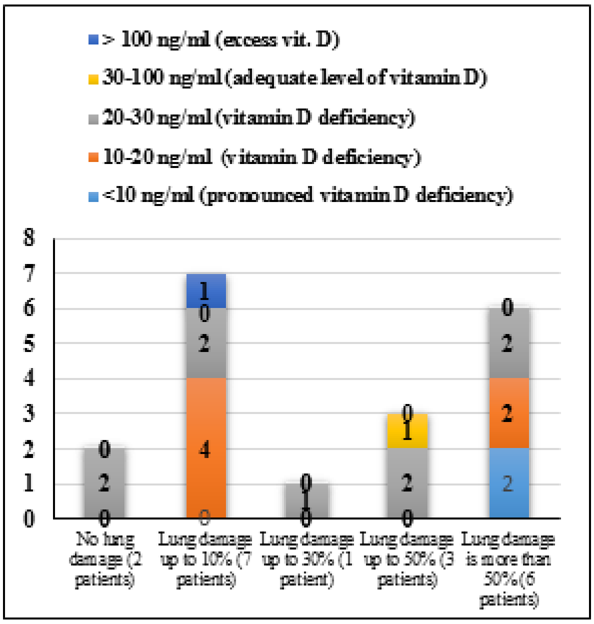


Fig. 6. Vitamin D status in the blood of the studied patients, depending on the degree of lung damage (19 patients in total)

The patients' blood levels of vitamin D with lung damage of more than 50% and, thus, severe clinical course of the disease showed critically low levels: of the six patients in this group, 2 had a pronounced vitamin D deficiency (<10 ng/ml), and another 2 had a vitamin D deficiency (10–20 ng/ml); the remaining 2 patients had blood levels of vitamin D insufficiency (20-30 ng/ml) (Fig. 6).

CONCLUSION

A retrospective analysis of laboratory-confirmed cases of COVID-19 in our study found a relationship between the blood level of 25-hydroxyvitamin D [25 (OH) D and the nature of the course of COVID-19 and the development of complications of the disease. Taking into account the main cause of critical complications of COVID-19 is impaired immunity, the systemic inflammatory response due to the "cytokine storm", and cholecalciferol harmonizes the functioning of the immune system at all its levels, it can be concluded that an adequate level of vitamin D in the blood increases the likelihood of a light course of COVID-19 and its favorable outcome. According to the literature, Vitamin D supplements are recommended for all infected and healthy people.

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Covid-19 xəstələrinin qaninda D vitaminin səviyyəsinin xəstəliyin qedişinə təsiri

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COVID-19 infeksiyasının təhlükəsi onun daha yüksək yoluxdurucu qabiliyyəti olmaqdadır (qripdən bir neçə dəfə yüksək), uzun bir inkubasiya müddəti (14 günə qədər) və heç bir simptomu olmayan xəstələrin infeksiya daşıyıcısı olması ilə əlaqələndirilir. Hal-hazırda, xəstəliyin xüsusiyyətlərinə dair klinik və epidemioloji tədqiqatlar, həmçinin qarşısının alınması və müalicəsi üçün yeni vasitələrin inkişafı davam edir. Ən vacib profilaktik vasitə antiviral immunitet sisteminin aktivləşdirilməsidir. Ədəbiyyat mənbələrinə əsasən, insanın orqanizmində D vitaminin yetərli tərkibi qrip virusuna qarşı da daxil olmaqla antiviral immunitet sisteminin əsaslarından biridir. COVID-19 olan xəstələrdə D vitamini səviyyələri ilə klinik nəticə arasında statistik baxımdan əhəmiyyətli məlumat yoxdur. Bu məqsədlə COVID-19-a yoluxan xəstələrin qanında D vitamini səviyyələrinin xəstəliyin gedişinə və müalicənin nəticələrinə təsirini öyrənmək olmuşdur. Laboratoriya tərəfindən təsdiqlənmiş COVID-19 infeksiyası olan 19 xəstənin xəstəlik tarixlərini təhlil etdik. Xəstələr 15.06.2020 - 15.07.2020 tarixləri arasında «MediClub» MMC-də müalicə alırdılar. «MediClub»

Xəstəxanası, COVIB-19 xəstələrinin müalicəsi üçün «TƏBIB» Azərbaycan tərəfindən təsdiq edilmişdir. 25-hidroksivitamin D[25(OH)D]-nin klinik təzahürləri və qan səviyyələri haqqında məlumatlar xəstəlik tarixlərindən əldə edilmişdir. Laborator təsdiqlənmiş COVID-19 hallarının retrospektiv analizi qanda 25-hidroksivitamin D[25(OH)D] səviyyəsi ilə koronavirus xəstəliyinin gedişatını və xəstəliyin fəsadlarının inkişafı arasındakı əlaqə barədə inamla danışmağa imkan verdi. COVID-19-un kritik ağırlaşmalarının əsas səbəbinin toxunulmazlığın zəifləməsi və xolekalsiferolun immunitet sisteminin işini bütün səviyyələrində uyğunlaşdırması olduğunu nəzərə alsaq, qanda kifayət qədər D vitamini səviyyəsinin COVID-19-un mülayim gedişatını və əlverişli nəticəsini artırdığı qənaətinə gəlmək olar.

Açar sözlər: *Koronavirus infeksiyası, pandemiya, COVID-19, 25-hidroksivitamin D[25(OH)D], D vitamininin qanda səviyyəsi, ağciyər zədələnməsinin həcmi, proinflamatuar sitokinlər, yanaşı xəstəliklər*

Влияние уровня витамина D в крови пациентов с COVID-19 на течение болезни

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Опасность заражения COVID-19 заключается в его более высокой контагиозности (в несколько раз выше, чем у гриппа), длительном инкубационном периоде (до 14 дней) и осложняется тем, что пациенты без каких-либо симптомов являются носителями инфекции. В настоящее время продолжаются клинико-эпидемиологические исследования характеристик заболевания, а также разработка новых средств его профилактики и лечения. Самый важный профилактический ресурс – это активация противовирусной врожденной иммунной системы. Опираясь на литературные данные, остаточное количество витамина D - одна из основ противовирусной иммунной системы, в том числе против вируса гриппа. Клинических испытаний для определения взаимосвязи между уровнем витамина D в крови и клиническим исходом у пациентов с COVID-19 не проводилось. Изучено влияние уровня витамина D в крови пациентов, инфицированных COVID-19, на течение заболевания и исход лечения. Проанализированы истории болезни 19 пациентов с лабораторно подтвержденной инфекцией COVID-19. Пациенты проходили лечение в ООО «MediClub» с 15.06.2020 по 15.07.2020. Госпиталь «MediClub» был одобрен «TABIB»Азербайджан для лечения пациентов с COVID-19. Данные о клинических проявлениях и уровнях гидроксивитамина D[25(OH)D] в крови были получены из историй болезни. Ретроспективный анализ лабораторно подтвержденных случаев COVID-19 позволил с уверенностью говорить о взаимосвязи между уровнем 25-гидроксивитамина D [25(OH)D] в крови, характером течения COVID-19 и развитием осложнений болезни. Учитывая, что основной причиной критических осложнений COVID-19 является нарушение иммунитета, а холекальциферол гармонизирует работу иммунной системы на всех ее уровнях, можно сделать вывод, что адекватный уровень витамина D в крови увеличивает вероятность легкого течения COVID-19 и его благоприятный исход.

Ключевые слова: *Коронавирусная инфекция, пандемия, COVID-19, 25-гидроксивитамин D[25(OH)D], уровень витамина D в крови, объем поражения легких, провоспалительные цитокины, сопутствующие заболевания*

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Importance of researching invasive species against the threat of future pandemics: the study of invasive plants in Azerbaijan

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The world is concerned about the COVID-19 pandemic. An active search is underway for ways to combat this threat. Invasions of plants and animals can be carriers of pathogens, themselves cause diseases and allergic reactions in humans. In recent decades, there has been an increase in their activity. The main reasons are intensive trade, population displacement and climate impacts. Once in a new environment, invasive species can multiply in large numbers. The researchers note that data on the early phases of invasions are extremely useful for understanding and predicting their distribution around the world. The identification of new alien species and the subsequent study of their influence on humans is one of the ways to combat their negative influences. Alien insects, birds, mammals, fungi and plants can become one of the reasons for the transfer of the pandemic, and their spread leads to its globalization. In Azerbaijan, in the study of invasions, as in the world, attention is paid to the problem of identification and registration of invasive species. This article presents some data on invasive plant species: the species that negatively affect human life are indicated, a comparative analysis of the content of the invasive fraction is carried out in the botanical- geographical regions of Azerbaijan.

Keywords: *Invasions and COVID-19, invasive flora of Azerbaijan, methods of investigating invasions, the impact of invasions on humans*

INTRODUCTION

Currently, we are living in a completely unusual regime associated with the worldwide spread of the COVID-19 disease, which has received the status of a pandemic. The problem of assessing and predicting the consequences of global invasions of alien species of flora and fauna in recent years and especially in 2020 comes to the fore (Palmer and Nursey-Bray, 2007; CBD Strategic Plan for Biodiversity 2011-2020, 2011). The decrease in the species diversity of ecosystems is due to the high competitiveness of invasions in comparison with local species (Van Kleunen et al., 2015). By occupying ecological niches and actively using soil minerals, changing light and water regimes they leave less resources for aboriginal species (Kunte, 2008; Hulme and Bremner, 2006). The growing activity of alien species is recognized not only as one of the factors of biodiversity loss, transformation of natural ecosystems, but also a threat to human health (Dyer et al., 2017; Pimental et al., 2001).

According to scientists, invasive insects, birds, mammals, fungi and plants are considered to be a special risk group in the transfer of diseases that threaten humans (Bertelsmeier and Olliver, 2020). It is expected that in the future arbovirus infections can be activated – carriers of which are arthropods, transmitting agents from host animals (e.g., birds), directly to humans (Weaver and Reisen, 2009). The animal world does not exist in isolation from the plant world. Therefore, in this chain, most likely, plants will be involved or possibly already involved - as vectors or as intermediate hosts. The scientific community has made progress in developing large checklists and databases on invasive species, for example, Delivering Alien Invasive Species Inventories for Europe (DAISIE),

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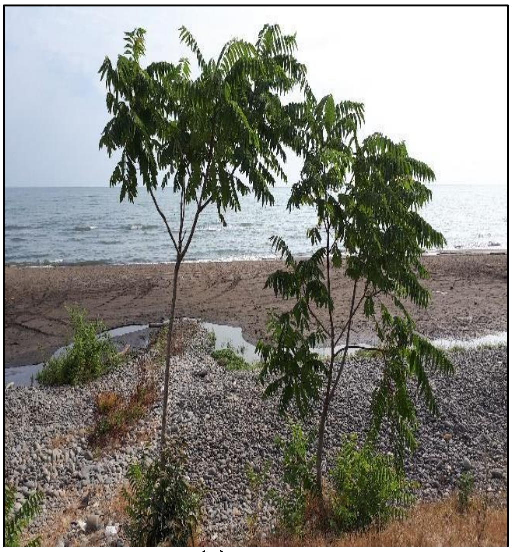
European Commission 2010, Global Invasive Species Database (GISD), IUCN, 2020, Global Register of Introduced and Invasive Species (GRIIS) IUCN, 2006], which allow researchers to map large-scale patterns of their intercontinental, inter-regional distribution (Turbelin et al., 2017).

The reduction of species diversity in the ecosystem leads to a high degree of competitiveness of the invasion by comparison with local species (Callaway and Aschchoug, 2000). According to the degree of exposure, alien species are classified into a number of groups, among which the most aggressive species are considered - transformes, which are able to change ecosystems in a significant area (Réjmánek et al., 2005). By occupying ecological niches and actively using soil minerals, changing light and water regimes, affecting allelopathically (Hierro and Callaway, 2003), etc., they leave less resources for aboriginal species. As a result, communities dominated by alien plants may include fewer native species. Among the invasive plants, there are species that have a negative effect on the human body (Fig. 1, a, b). In particular, in some regions of Azerbaijan there are *Ambrosia artemisiifolia* L., *Ailanthus altissima* (Mill.) Swingle, *Euphorbia humifusa* Schlecht., *Euphorbia maculata* L.causing allergic reactions in humans.

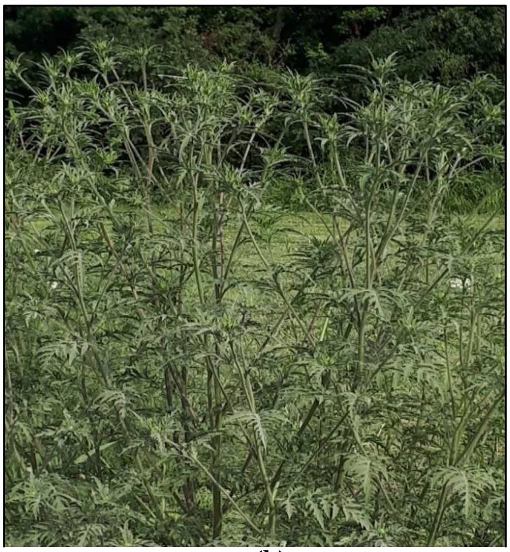
Invasive and expansive species *Ailanthus altissima* is observed in all regions of Azerbaijan, including coastal areas. There are also invasive species *Xanthium strumarium* L., *X. spinozum*L., *Amaranthus retroflexus* L., *Chenopodium album* L., *Coniza canadensis* L. and etc. which play a dominant role in vegetation. Species- *Acalypha australis* L., *Erigeron annuus* (L.) Pers. which are rapidly spreading in certain localities have been also determined. New alien species *Oenothera odorata* Jacq.has been also identified for the flora of Azerbaijan (Abdiyeva et al., 2020).

Currently, there are a number of approaches and directions in the study of invasive species - identification of the mechanisms of their introduction and distribution, the study of biological, phytocenotic features, allelopathic influences; genetic and genomic approaches, as well as the ecological approach to studying the activity of invasions in connection with climatic changes, the search for natural enemies - phytophagan and vice versa, various microorganisms and fungi that are in symbiosis with alien plants, etc. are also developing .

The main methods of studying invasions include an effective strategy for controlling invasive species, including the creation of various databases (DAISIE), ecological and geographical modeling of invasion niches, allowing for high-precision determination of their distribution, predicting the state and further spread of invasions by computer modeling methods using involvement of GIS technologies.



(a)



(b)

Fig. 1. (a) *Ailanthus altissima;* (b) *Ambrosia artemisiifolia.*

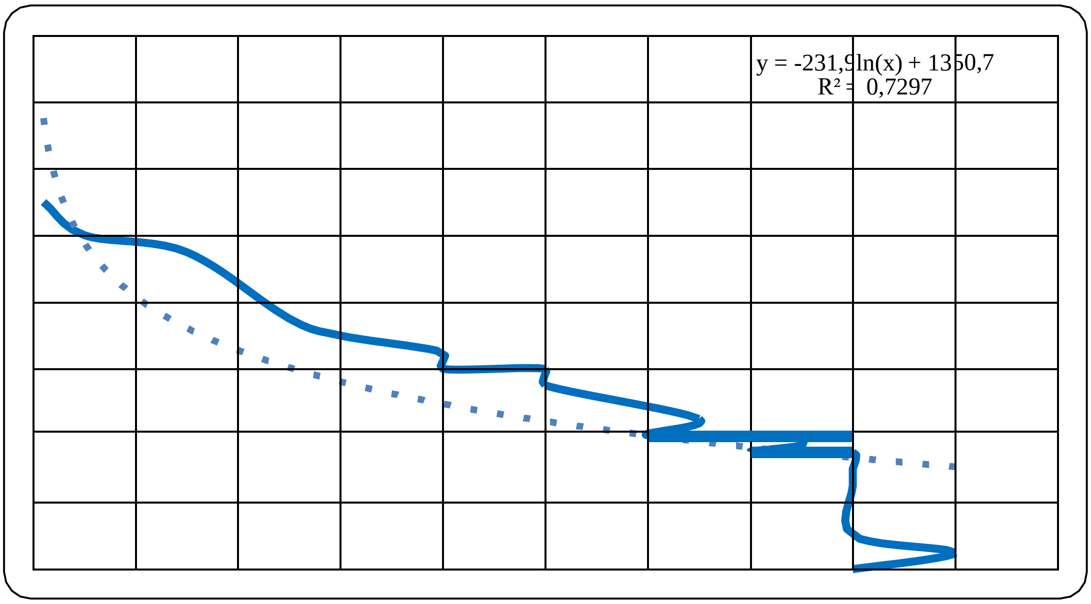


Fig. 2. Distribution of alien species depending on the height above sea level
(y-axis - height above sea level (m), x-axis - occurrence of species (%)).

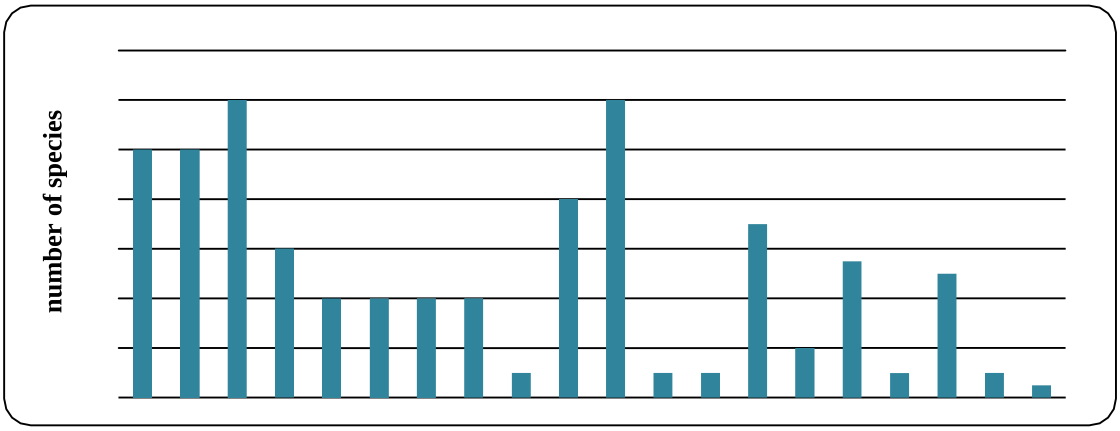


Fig. 3. The ratio of the fractions of the alien flora in the botanical-geographical regions of Azerbaijan.

At present, the Institute of Botany of ANAS, taking into account the above-mentioned approaches and methods, is conducting special studies of invasive plant species in Azerbaijan - a preliminary "black list" of these species has been drawn up, numbering 64 species belonging to 22 families and 46 genera (Abdiyeva, 2018), which will most likely be replenished as our research expands. Established the distribution of alien flora is subject to vertical zoning. The optimal height for the growth of invasions is in the range of 100-600 (700) m a.s.l. (Fig.2). Studies show that the species diversity of plants and the territories of their natural habitats are increasingly subject to global climate change and anthropogenic impact. As a result, there is a change in the structure of the vegetation cover and its fragmentation, as well as a decrease in the number of populations of most characteristic species and a progressive activity of alien plants entering into interspecific competition with native plants.

At the same time, the key places of localization of invasive plants are areas with the cultivation of cotton, tea, rice and tobacco. This is the central part of the Kura - Araz lowland, the Lankaran

group of districts, as well as the northern regions of the country (Fig. 3). The Institute has begun modeling the geographical limits of the spread of invasive plant species on the territory of Azerbaijan due to climate change.

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Gələcək pandemiyanın təhlükəsindən invaziv növlərinin araşdırılmasının əhəmiyyəti: Azərbaycanda invaziv bitki növlərinin öyrənilməsi

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Dünya COVID-19 nəticəsində yaranan pandemiyadan olduqca narahatdır. Bu təhlükə ilə mübarizə yolları üçün fəal çıxış yolları axtarılır. Bitki və heyvanların invaziyası xəstəliklərin yaranmasının daşıyıcıları ola bilər və ya özləri insanlarda xəstəliklərə və allergik reaksiyalara səbəb olur. Son onilliklərdə onların sürətli artımı müşahidə edilir. Buna əsas səbəblər - intensiv ticarət, əhalinin yerdəyişməsi və iqlimə olan təsir

hesab oluna bilər. Yeni bir yaşama mühitində invaziv növlər çoxlu sayda arta bilərlər. Alimlər tərəfindən qeyd olunur ki, invaziyanın ilkin mərhələlərində toplanılan məlumatlar, onların dünyada yayılmasının tədqiqində və proqnozlaşdırılmasında olduqca zəruridir. Yeni yad növlərin müəyyənləşdirilməsi və onların insanlara təsirinin tədqiqi onların mənfi təsirləri ilə mübarizə yollarından biridir. Yad həşəratlar, quşlar, məməlilər, göbələklər və bitkilər qloballaşmanın bir nəticəsidir və COVID-19 pandemiyası kimi qlobal bir həll tələb edir. Dünyada olduğu kimi, Azərbaycanda da invaziyaların tədqiqi baxımından invaziv növlərin probleminə olduqca diqqət yetirilir.

Açar sözlər: *İnvaziyalar və COVID-19, Azərbaycanın invaziv florası, invaziv növlərinin öyrənilməsi metodları, invaziv növlərin insanlara təsiri*

Важность исследования инвазивных видов от угрозы пандемий в будущем: изучение инвазивных видов растений в Азербайджане

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Мир обеспокоен пандемией, вызванной COVID-19. В настоящее время проводится активный поиск путей борьбы с этой угрозой. Инвазивные растения и животные могут являться переносчиками возбудителей заболеваний, а также сами вызывать заболевания и аллергические реакции у человека. В последние десятилетия наблюдается рост их активности. Основные причины - интенсивная торговля, перемещение населения и воздействие на климат. Оказавшись в новой среде инвазивные виды, могут размножаться в больших количествах. Исследователи отмечают, что данные о ранних фазах вторжения инвазий чрезвычайно полезны для понимания и прогнозирования их распространения по всему миру. Выявление новых заносных видов и последующее изучение их влияния на человека является одним из путей борьбы с их негативными влияниями. Заносные насекомые, птицы, млекопитающие, грибы и растения являются результатом глобализации и требуют глобальных ответных мер, как и пандемия COVID-19. В Азербайджане, как и во всем мире, большое внимание уделяется проблеме заносных видов с точки зрения изучения инвазий.

Ключевые слова: *Инвазии и COVID-19, инвазивная флора Азербайджана, методы исследования инвазий, влияние инвазий на человека*

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A time-dependent *SIR* model for COVID-19 in Azerbaijan

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A novel coronavirus named ''2019-nCoV'', has been causing the deadliest pandemic in late 2019 and early 2020. This novel virus was defined as the coronavirus disease 2019 (COVID-19) by the World Health Organization (WHO). Diseases have afflicted humans ever since there have been human beings. From AD 541 to 542, the global pandemic known as "the Plague of Justinian" is one of the worst pandemics in the world and is estimated to have killed 15–25% of the world's 200 million population. Today we are battling to control and prevent the spread of COVID-19. Coronavirus has the potential to cause the deadliest pandemic in human history. The number of cases of COVID-19 outside China has drastically grown up since 16th March, 2020. On 28 February, 2020 Azerbaijan has confirmed first positive case of COVID-19 within its border. The patient, a Russian national, had traveled from Iran to Azerbaijan. On 31 October, 2020 the total number of confirmed coronavirus cases is 55.269 in Azerbaijan. In this paper, we conduct mathematical and numerical analyses of COVID-19. We have applied the SIR model considering data from Azerbaijan. Assuming the published data are reliable, the SIR model can be applied to assess the spread of the COVID-19 disease and predict the number of infected, removed and recovered populations and deaths in the communities, accommodating at the same time possible surges in the number of susceptible individuals.

Keywords: *COVID-19, SIR, mathematical model, simulation, susceptible, infected, recovered*

INTRODUCTION

Due to the comparable transmissibility as severe acute respiratory syndrome coronavirus (SARS-CoV) in 2003, since the first case of corona virus disease 2019 (COVID-19) was reported in Wuhan city of China in late December 2019, it quickly spread to 24 countries in 4 continents around the world in less than two months. On January 30, the World Health Organization (WHO) declared this fast-growing outbreak of COVID-19 as a Public Health Emergency of International Concern (PHEIC) (Tang et al., 2020).

COVID-19 has spread rapidly worldwide, and World Health Organization (WHO) has increased the assessment of the risk of spread and risk of impact of COVID-19 to very high at the global level and has labeled it as a "pandemic". Therefore, it becomes necessary to develop and use the mathematical approach to study the pathogenesis of this virus in humans.

Daily updated data of COVID-19 in countries outside China were collected from the coronavirus disease (COVID-2019) situation reports released by WHO (Wu et al., 2020a).

Due to the continuous public health interventions adopted in China and other countries outside China, the transmission model of COVID-19 would change all the time until it arrived at a relatively stable status (Wu et al., 2020b). Therefore, the time-varying SIR models were developed based on the daily increased case number and were used to calculate the infection parameters of the COVID-19.

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MATERIALS AND METHODS

In the typical mathematical model of infectious disease, one often simplify the virus-host interaction and the evolution of an epidemic into a few basic disease states. One of the simplest mathematical models of disease spread is SIR (Susceptibles, Infectives, Removed) model (Tang et al., 2020). It splits the population into three basic categories according to disease status.

People who have not yet had the disease are labelled "susceptibles". Everyone is assumed to be born susceptible and they capable of being infected.

Those who have infected the disease and are capable of passing it to susceptibles are the "infectives". The third group are euphemistically referred to as the "removed" class. These are the people who have had the disease and recovered or those who have died. This is referred to as the SIR model. SIR model can provide information for how to prevent diseases spreading.

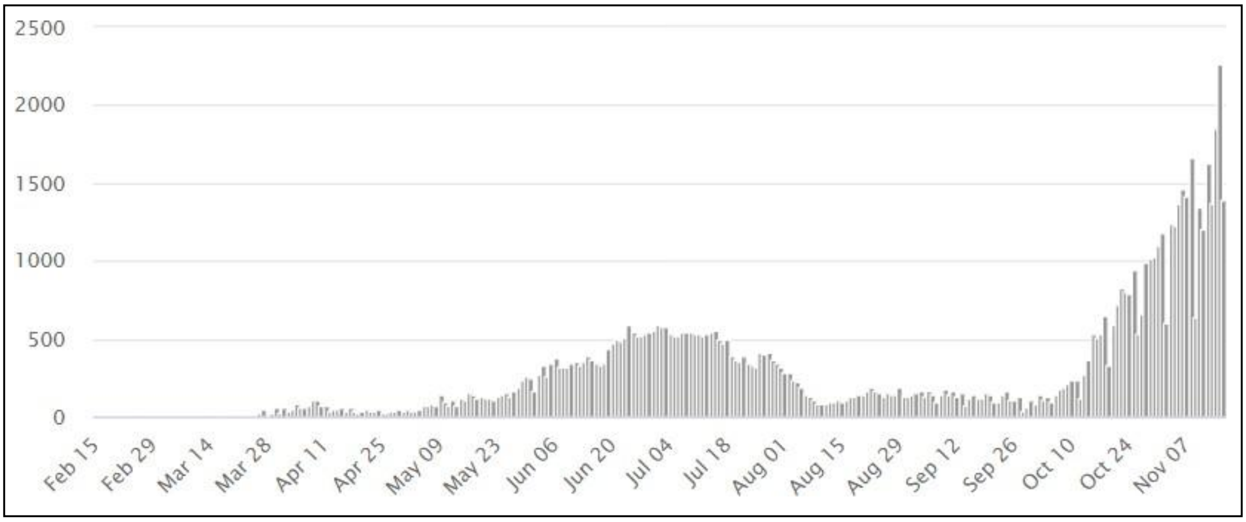


Fig. 1. Daily new cases in Azerbaijan.

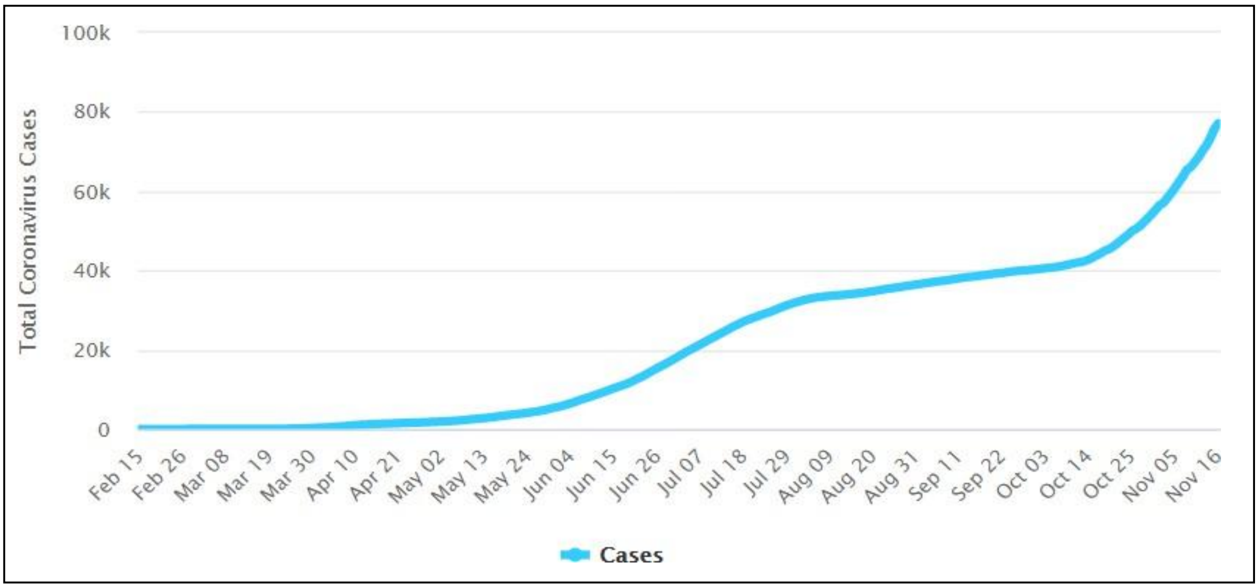


Fig. 2. Total coronavirus cases in Azerbaijan.

In the traditional SIR model, it has two time-invariant variables: the transmission rate and the recovering rate (Kraemer et al., 2020). The transmission rate means that each individual has on average contacts with randomly chosen others per unit time (Nishiura et al., 2020). On the other hand, the recovering rate indicates that individuals in the infected state get recovered or die at a fixed average rate. This assumption is too simple to precisely and effectively predict the trend of the disease.

$$\frac{dS}{dt} = -\beta IS \quad \textbf{(1)}$$

$$\frac{dI}{dt} = \beta IS - \gamma I \quad \textbf{(2)}$$

$$\frac{dR}{dt} = \gamma I \quad \textbf{(3)}$$

Therefore, we propose the time-dependent SIR model, where both the transmission rate and the recovering rate are functions of time t (Wangping et al., 2020). Such a time-dependent SIR model is much better to track the disease spread, control, and predict the future trend (Tang et al., 2020).

As in the classical SIR model, S(t), I(t), R(t) represented the number of susceptible, infectious, and recovered people respectively at time t in the population size of N (Scarpino and Petri, 2019). To model the dynamics of the outbreak we need three differential equations, one for the change in each group, where β and γ represented the probability of a susceptible-infected contact resulting in a new infection and the probability of an infected case recovering and moving into the resistant phase, respectively (Chinazzi et al., 2020).

In these equations, the parameters β (the infection rate) and γ (the recovery or removal rate of infectives) (Wu et al., 2020) are constants: β controls the transition between S and I, equation (1), while γ controls the transition between I and R, equation (3) (Rüdiger et al., 2020). From a dimensional point of view, assigning no units to S, I, R, and N the parameters β and γ have units of inverse of time (measured typically in days, weeks or months in epidemiological records) (Kucharski et al., 2020).

Notice that equation (1) expresses the interaction between S and I (at time t) as the product SI and that a fraction of this product are the individuals that at time t becomes infected and removed from S (which, because of the negative sign in equation (1), decreases as time increases from zero) (Yang et al., 2020). This interaction in the form of the product SI makes difficult to determine the parameter β from observed epidemiological data. On the other hand, from equation (3), the inverse of the parameter (γ) gives a measure of the time spent by individuals in the infectious state (Fanelli and Piazza, 2020). Consequently, by carefully observing the development of an infectious disease, the parameter γ can be estimated relatively precisely by epidemiologists from epidemiological records (as the inverse of the recovered or infectious period) (Hethcote, 2009).

Beta is the infection rate of the pathogen, and gamma is the recovery rate. They are real, positive, parameters of the initial exponential growth and final exponential decay of the infected population *I* (Giordano et al., 2020). Together, these two values give the basic reproduction number R_0. Basic reproduction number (R_0) is avarage number of people who will catch the disease from single infected person. If the R_0 value is greater than one, the infection rate is greater than the recovery rate, and thus the infection will grow throughout the population (Jung et al., 2020). If R_0 is less than one, the infection quickly will die out since people are healing faster than they are spreading it. Basic reproduction number (R_0) for COVID-19 is 1.4-5.7.

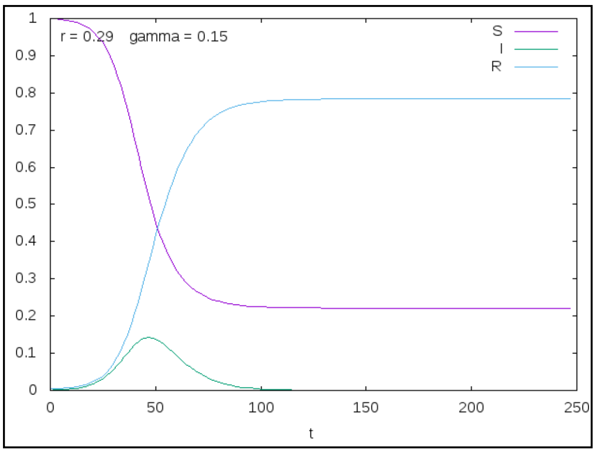


Fig. 3. Simulation of the SIR model of the spread of a disease in Azerbaijan.

Based on these assumptions and concepts, the rates of change of the three populations are governed by the following equation of SIR, what constitutes the SIR model used in this study (Backer et al., 2020).

From the simulation of the SIR model, the first day starts with only one infected person then the infection rate in the simulation increases exponentially with the increasing number of new infected people.

CONCLUSION

The rate of increase in the number of infections depends on the product of the number of infected and susceptible individuals. An understanding of the SIR simulation explains the staggering increase in the infection rate in the Azerbaijan. Infected people traveling different parts of the Azerbaijan and has led to the increase in infected numbers and this results in a further increase in the susceptible population. This gives rise to a positive feedback loop leading to a very rapid rise in the number of active infected cases.

Thus, during this period, the number of susceptible individuals increases and as a result, the number of infected individuals increases as well. For example, as of 19 May, 2020, there were 3518 infected individuals and by 31 October, 2020, this number had grown to a staggering 55,269.

Here, we have applied the SIR model considering data from Azerbaijan. Assuming the published data are reliable, the SIR model can be applied to assess the spread of the COVID-19 disease and predict the number of infected, removed and recovered populations and deaths in the communities, accommodating at the same time possible surges in the number of susceptible individuals.

The countries in the world took extreme actions with closures, confinement, social distancing, and people wearing masks. This type of action produces a decline in the number of infections and susceptible individuals. If the number of susceptible individuals does not decrease, then the number of infections just gets increased rapidly.

As at this moment, there is no effective vaccine developed, the only way to reduce the number of infections is to reduce the number of individuals that are susceptible to the disease. Consequently, the rate of infection tends to zero only if the susceptible population goes to zero.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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COVID-19-un Azərbaycanda zamandan asılı SİR modeli

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“2019-nCoV” adlanan yeni bir koronavirus 2019-cu ilin sonu və 2020-ci ilin əvvəllərində ən ölümcül pandemiyaya səbəb olur. Eramızdan əvvəl 541-542 arasında "Justinianın taunu" olaraq bilinən qlobal pandemiya dünyanın ən pis pandemiyasından biridir və dünyanın 200 milyon əhalisinin 15-25% -ni öldürdüyü təxmin edilir. Bu gün COVID-19-un yayılmasının qarşısını almaq üçün mübarizə aparırıq. Koronavirus insanlıq tarixində ən ölümcül pandemiyaya səbəb ola bilər. Çin xaricində COVID-19 hadisələrinin sayı 16 Mart 2020-ci ildən bəri kəskin şəkildə artmışdır. 28 fevral 2020-ci ildə Azərbaycan, sərhədində ilk müsbət COVID-19 hadisəsini təsdiqlədi. Rusiya vətəndaşı olan xəstə İrandan Azərbaycana səyahət etmişdi. 31 oktyabr 2020-ci il tarixdə Azərbaycanda təsdiqlənmiş koronavirüs hadisələrinin ümumi sayı 55269-dur. Bu məqalədə COVID-19-un riyazi və ədədi analizlərini aparırıq. Azərbaycandan alınan məlumatları nəzərə alaraq SIR modelini tətbiq etdik. Nəşr olunan məlumatların etibarlı olduğunu düşündükdə, SIR modeli COVID-19 xəstəliyinin yayılmasını proqnozlaşdırmaq üçün tətbiq oluna bilər.

Açar sözlər: *COVID-19, SİR, riyazi model, simulyasiya, həssaslar, yoluxmuşlar, bərpa olunmuşlar*

Времязависимая модель *SIR* COVID-19 в Азербайджане

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Новый коронавирус, названный «2019-nCoV», вызвал самую смертоносную пандемию в конце 2019 - начале 2020 года. Этот новый вирус определен Всемирной организацией здравоохранения (ВОЗ) как коронавирусное заболевание 2019 года (COVID-19). Болезни поражают людей со времен их появления. С 541 по 542 годы нашей эры глобальная пандемия, известная как «Чума Юстиниана», была одной из самых страшных пандемий и, по оценкам, унесла жизни 15–25% 200-миллионного населения мира. Сегодня мы ведем борьбу за контроль и предотвращение распространения COVID-19. Коронавирус может вызвать самую смертоносную пандемию в истории человечества. Число случаев COVID-19 за пределами Китая резко выросло с 16 марта 2020 года. 28 февраля 2020 года на территории Азербайджане был выявлен и подтвержден первый положительный случай COVID-19. Пациентом оказался гражданин России, приехавший из Ирана в Азербайджан. На 31 октября 2020 года общее число подтвержденных случаев коронавируса в Азербайджане составило 55269 человек. В этой статье мы проводим математический и численный анализ распространения COVID-19. Мы применили модель SIR с учетом данных из Азербайджана. Предполагая, что опубликованные данные надежны, модель SIR может быть применена для оценки распространения болезни COVID-19 и прогнозирования результата.

Ключевые слова: *COVID-19, SIR, математическая модель, симуляция, чувствительный, инфицированный, восстановленный*